Master's Dissertation

SIMULATION MODEL OF BACTERIAL RESISTANCE TO ANTIBIOTICS

박테리아의 항생제 내성에 대한
시뮬레이션 모델 연구

August 2015

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ABSTRACT

Simulation Model of Bacterial Resistance to Antibiotics

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Antibiotic resistance or antimicrobial resistance (AMR) refers to infections caused by bacteria, fungi, parasites and viruses resistant to antibiotics. Antibiotic resistance has become a major public health threat as it breaks out regardless of geographical conditions or socioeconomic status. Infectious diseases such as HIV/AIDS, Tuberculosis, Malaria, etc., are known to be more widely spread in developing countries compared to other developed countries. Experts from various fields are putting their effort to tackle this problem however is yet to be solved. Various computational approaches including bioinformatics have been conducted such as genome sequencing, constructing databases of resistance genes and antibiotics information, providing tools for analysis and designing simulation models. The purpose of this study is to design and implement simulation models of bacterial growth and antibiotic resistance to find the proper antibiotics against antibiotic resistant bacteria. Simulation models were designed based on Individual based Modeling (IbM). A simulation tool named ARSim was developed in order to conduct experiments using simulation models. We designed models on top of ARSim to observe the growth of bacteria and predict the consequences of

adding antibiotics into the bacterial population. Simulations of bacterial growth were conducted by growing *K.pneumoniae* bacteria on a virtual plate with predefined parameters. By assessing the change in bacterial population as time goes by, the result was nearly identical to the four phases of a bacterial growth curve. The next experiment was predicting the effects of antibiotics when added to two different groups, one group of non-resistant bacteria and another group of both resistant and non-resistant bacteria. We assumed carbapenem class Imipenem and Meropenem as antibiotics and carbapenem resistant bacteria as the bacterial strain. In the first experiment, we predicted that the non-resistant bacterial population steadily grows when 0.05μg/ml of Imipenem is added to the population. On the contrary the population instantly died out when 0.1μg/ml was added which is greater than the minimum inhibitory concentration of the strain. In the second experiment, we added Imipenem and Meropenem with concentrations of 16μg/ml, 32μg/ml and 64μg/ml each. The results for adding Imipenems were akin to previous lab experiments in literature and results for Meropenems were very much alike to Imipenems. We used Individual based Modeling methods to design and implement models of bacteria, antibiotics, enzymes and the environment and conducted simulations of these entities through the ARSim program. Results were shown that properties and interactions among these entities were properly defined, and the models to a certain degree follow the biological principles of bacteria and their mechanisms of antibiotic resistance. Using the computational approaches made in this study, we hope to provide researchers with a better option on finding new ways of fighting antibiotic resistance.

Keywords : Antibiotic resistance, Antibiotic Resistant Bacteria, Superbugs, Bioinformatics, Individual based Modeling, Simulation models

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Sincere appreciation to my advisor for his guidance
and my beloved family for their support

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LIST OF ABBREVIATIONS

AbM	Agent based Modeling
CDC	Center for Disease Control and Prevention
CRE	Carbapanem-resistant Enteriobacteriaceae
EARS-Net	European Antimicrobial Resistance Surveillance Network
ESBL	Extended-spectrum beta-lactamase
IbM	Individual based Modeling
JSON	JavaScript Object Notation
JVM	Java Virtual Machine
KPC-1	*Klebsiella pneumoniae* carbapenemase 1
MIC	Minimum Inhibitory Concentration
NDM-1	New Delhi metallo-beta-lactamase 1
NIH	National Institutes of Health
PBP	Penicillin binding proteins
WGS	Whole Genome Sequencing

CHAPTER I.

INTRODUCTION

1.1 Background

Antibiotic resistance or antimicrobial resistance (AMR), becoming one of the increasing public health threats, refers to infections caused by bacteria, fungi, parasites and viruses resistant to antibiotics. Standard treatments become ineffective to these kinds of resistant microorganisms, increasing the risk of spread to others (WHO, 2014). Unlike infectious diseases such as HIV/AIDS, Tuberculosis, Malaria spread widely in many developing countries (Fonkwo, 2008), antibiotic resistance breaks out irrespectively of a country's geographical conditions or socioeconomic status, which worsens the severity of antibiotic resistance as a public health threat. Especially, as antibiotic resistance can be acquired not only from medical institutions (HAI; hospital-acquired infection) but also from the community (CAI; community-acquired infection), it becomes increasingly difficult to tackle antibiotic resistance due to its various infection routes.

According to the United States CDC (Centers for Disease Control and Prevention), the major cause for the dramatic increase in antibiotic resistance was bacteria acquiring resistance genes, mainly due to the imprudent use of antibiotics. Reports have shown that approximately 50% of antibiotic prescriptions were excessive or unnecessary (US CDC, 2013) and antibiotic use in livestock for growth enhancement and feed efficiency is providing routes to bacterial infections in human.

Public health agencies worldwide publish annual reports regarding the statistics and trends of antibiotic resistance. In the United States, CDC releases reports on antibiotic resistance and classifies antibiotic resistant bacteria into three levels depending on their severeness to people's health and healthcare expenditures. These microorganisms are classified as 'Urgent', 'Serious' and 'Concerning', and 18 kinds of infections are dealt with in the report. In a report in 2013, accumulation of more than 2 million bacterial infections were counted and 23,000 of those who were infected died, leading to 55 trillion dollars of economic loss due to the increase of healthcare and social expenditures (Table 1.1). In Europe, EARS-Net (European Antimicrobial Resistance Surveillance Network) under ECDC (ECDC: European Centre for Disease Prevention and Control) published reports annually from 2001 and a recent report has shown that more than 3 million infections had been occurred in 2011. EARS-Net provides more detailed information by categorizing the origin of infections inside a medical facility to ICU, OR, ward etc. The WHO also publishes reports annually, starting from 2001 when the organization set up a guideline to prevent the spread of antibiotic resistance infections. This report gives a glimpse on the global trend of antibiotic resistance by collecting and organizing data from countries and institutions worldwide. Being late than other countries, South Korea started to monitor antibiotic resistant bacterial infections from 2010. South Korea designated 6 major antibiotic resistant bacterial species and established guidelines for healthcare institutions to easily report various outbreaks to the government (South Korea CDC, 2012). Total number of infections has been rapidly rising in South Korea, from 22,928 infections in 2011 to 44,174 in 2012 and 80,955 in 2013, nearly doubling every year (Figure 1.1) (South Korea CDC, 2014).

The rapid increase in antibiotic resistance has been drawing public attention over the years but interventions for this public health phenomenon

are still unsatisfactory. As we are in desperate need of effective ways, which includes research and establishing guidelines or policies, to prevent the spread of antibiotic resistance, using computer simulation models in bacteria and antibiotic resistance will be able to provide more efficient ways to perform antibiotic resistance research.

1.1.1 Antibiotics

Antibiotics or antibacterials are substances made from microorganisms that are used for treating bacterial infections, destroying or inhibiting the growth of bacteria by their various mechanisms of action. Antibiotics are prescribed by their classification of action mechanisms or antibacterial spectrum (Boundless, 2015). Bacteria being affected are divided into categories such as Gram-positive or Gram-negative by their Gram staining, aerotropic or anaerobic by their necessity of oxygen, Bacillus or coccus by their form. Mechanisms of actions include interference with cell wall synthesis, interference with cell membrane permeability, interference with protein, DNA or nucleic acid synthesis (National Health Information Portal, Ministry of Health and Welfare, South Korea).

The most common antibiotics that interfere with or inhibits cell wall synthesis are beta-lactam antibiotics such as Penicillins, Carbapenems, Monobactams or Glycopeptide antibiotics such as Vancomycin. These antibiotic molecules bind to PBP, also known as Penecillin binding proteins which are needed for a cell wall synthesis, consequently weakening the cell walls and forces bacteria to burst by osmotic pressure (Kang, 2004). Another mechanism is interfering with cell membrane permeability, most commonly known as Polymyxins. These antibiotics interact with phospholipids in bacterial cell membrane, forcing the hydrophobic tail to cause damage to the outer and inner membranes. Mechanisms that inhibit protein synthesis are done by suppressing the production of mRNA, interfering with the binding

between tRNA and mRNA or the peptide bond. Since human ribosomes are different from bacterial ribosomes, this mechanism minimizes side effects which may occur. Antibiotics that inhibit nucleic acid synthesis first interfere with the transcription process from the DNA which leads to failure in creating RNAs. Lastly, antibiotics that inhibit folic acid synthesis induce bacteria to folic acid deficiency by interfering with the synthesizing process of folic acids in the bacteria.

1.1.2 Antibiotic resistant bacteria

Patients must properly use antibiotics according to a prescription, both the right dosage and period. Misuse or overuse of antibiotics may lead to acquirement or increase of resistance genes in the bacterial population. The NIAID (National Institute of Allergy and Infectious Disease) under NIH (National Institutes of Health) classifies the spread and transfer of antibiotic resistance into two main causes, biological and non-biological.

The first biological cause is the elimination of all bacteria including bacteria without antibiotic resistance when using antibiotics, naturally increasing the portion of antibiotic resistant bacteria within the population. Second is the mutation in genes when bacteria are exposed to a certain dose of antibiotics. The third factor is conjugation when genes from adjacent bacteria are transferred horizontally into the plasmid. There also exists a method using viruses called bacteriophages where these bacteriophages acquires resistance genes from one bacterium and moves them to another.

Causes other than biological factors include the abuse of antibiotics, insufficient diagnosis and improper prescriptions, using healthcare facilities and the overuse of antibiotics in livestock. In spite of the unnecessariness of antibiotic use in viral infections such as common cold, influenza, otitis media

or rash, continuous use of antibacterial agents may develop drug resistance to bacteria such as *Staphylococcus aureus, Streptococcus pneumonia*, resulting in fatal conditions (Hildreth et al., 2009). Also, it is estimated that more than 720,000 antibiotic resistance infection cases in 2011 were acquired from healthcare facilities which means that just visiting these facilities increases exposure to fatal bacteria and may lead to infection (Magill et al., 2011).

According to reports from the agricultural and livestock industries, more than 50% of antibiotics produced in the United States were consumed in these two industries (National Research Council, Committee on Drug Use in Food Animals, 1999; Mellon et al., 2001). Animals over-exposed to antibiotics have greater chance of developing antibiotic resistance, consequently contaminating water, crops and grain with feces and urine.

There are four common ways of antibiotic resistant bacteria to block antibiotic actions (Figure 1.2) (Walsh, 2000). First, bacteria exposed to antibiotics synthesize particular enzymes that inactivate antibiotic molecules activities. When antibiotic agents permeate into the bacterial cell, a resistance gene enables the production of enzymes, for example a beta-lactamase, which eventually blocks the effect of antibiotics. The second method is transforming the target of antibiotic actions which includes how PBPs are transformed when resistant bacteria react with Penicillin. The next method is blocking the antibiotic agents from permeating into the cell membrane and lastly, bringing the agents which have already entered the cell out of the membrane which is also called the efflux mechanism. Bacteria are able to gain resistance to antibiotics through the above four mechanisms and two or more mechanisms can be applied simultaneously.

1.1.3 Bioinformatics approaches

Various computational approaches including bioinformatics have been put in antibiotic resistance research. Through technological advances in sequencing along with lowered costs, WGS (Whole Genome Sequencing) methods have been rising to gain influence in genome sequence analysis over traditional laboratory experiment methods such as PCR or microarrays. Bioinformatics methods were utilized in a research where vast amount of samples from urine were quickly analyzed through whole genome sequencing compared to traditional methods, resulting in finding numerous unknown resistance gene that have not yet been found in any other experiments (Hasman et al., 2013). There have also been studies where 3782 antimicrobial peptides were collected and analyzed. As a result, features were extracted and predictions were made by developing an integrated system with database e(Thomas et al., 2010). Similarly, studies were conducted where antimicrobial peptide sequences were analyzed using the Hidden Markov model to find the appropriate candidate substances of antimicrobial peptides (Fjell et al., 2007).

Other computational methods include providing useful information to researchers by constructing databases. In order to construct databases, you first need to gather data from public websites and arrange the data to retrieve useful information. In a study where a database of antibiotic resistance was constructed, data were gathered and classified into antibiotics, antibiotic resistance gene profiles, ontologies and annotations (Liu and Pop, 2009). A similar study was held by constructing a database of antibiotic resistance genes, gene profiles, resistance mechanisms, ontologies and annotation and providing bioinformatics methods to analyze the data (Fjell et al., 2007). It is worth constructing databases as a strong basis of developing an integrated resource center that provides not only genetic data but also features for analyzing and processing these data into useful information (Wattam et al.,

2014).

1.1.4. Simulation methods

While most studies in the area of bioinformatics are focused on sequence analysis and database construction, there exist studies that involve prediction and estimation of antibiotic resistance by using simulation methods. Simulation methods include modeling antibiotic resistance for effective control and intervention to a clinical or public health perspective, or modeling the biological systems of bacteria and their interactions with antibiotics, where the idea is to make observations and predictions from a biological perspective.

The first method include modeling antibiotic resistance for analyzing the pathways in the spread of antibiotic resistance in clinical settings (Sébille and Valleron, 1997; Weinstein et al., 2001; Haber et al., 2010), using computational models for treatment control in hospitals (MC D'Agata et al., 2007), and expanding the use of mathematical models to community acquired antibiotic resistance (Austin and Anderson, 1999). These approaches have in common that models are designed based on data from medical records from patients in real world hospital environment. The aim is to assess the current status of antibiotic resistance in hospitals to control this issue, or come up with an effective treatment protocol to avoid antibiotic resistance.

The second method mentioned above is a computational biology or systems biology approach, where biological mechanisms are modeled for computation. By creating a virtual environment with virtual cells, we are able to monitor the growth of bacteria and perform virtual experiments by adding various parameters (Tomita et al, 1999). For these virtual experiments, or simulations, mathematical models that describe the change in number of cells during bacterial growth must be implemented in a computer simulation

program (Gregory et al., 2008; Murphy et al., 2008). Also, similar researches in this field include describing the process of molecular actions by modeling and implementing the biochemical pathways beneath (Autiero et al., 2009).

There are two types of modeling methods categorized by their flow of simulation. One is the top-down modeling approach where there exist global rules that affect all agents of the model and characteristics of individual cells in the bacterial population are completely excluded. This model is also known as the Population model and using the Logistic growth model is the most popular technique to implement the Population model. In the Logistic growth model, a variable named carrying capacity is set as the maximum number of cells the model can hold in its environment. Once the variable is set, we are able to track the changes of bacterial growth in real time and compare the results with the numbers derived from laboratory experiments (Grimm, 1999; Nykamp). The biggest advantage using top-down modeling in simulations is the relatively simple and inexpensive computation. This means that small amounts of resources are needed to implement and conduct experiments. On the other hand, this simple model is insufficient in real world applications such as modeling the relationship and interactions between bacteria and antibiotics. A more complex model is needed to explain the actions not only between bacteria and antibiotics, but also between each bacterial cells or enzymes. The bottom-up approach has advantages in performing simulations similar to real world settings by modeling individual characteristics and interactions of bacteria within the population. Individual based Modeling (IbM) or Agent-based Modeling (AbM) is typically used for top-down modeling approaches (Gorochowski et al., 2012). These types of approaches can also be referred to as Rule-based approach since rules reflecting the mechanisms are set for all entities in the model (Vlachosa et al., 2006; Michel et al., 2009). In IbMs, parameters are set to each agent making up the model and heterogeneous factors of agents are all applied into the simulation (Makal

et al, 2006). In this study, each bacterium in the model had to maintain different characteristics such as its resistance to antibiotics, cell size etc., so we concluded that IbM was the appropriate method for our simulation model.

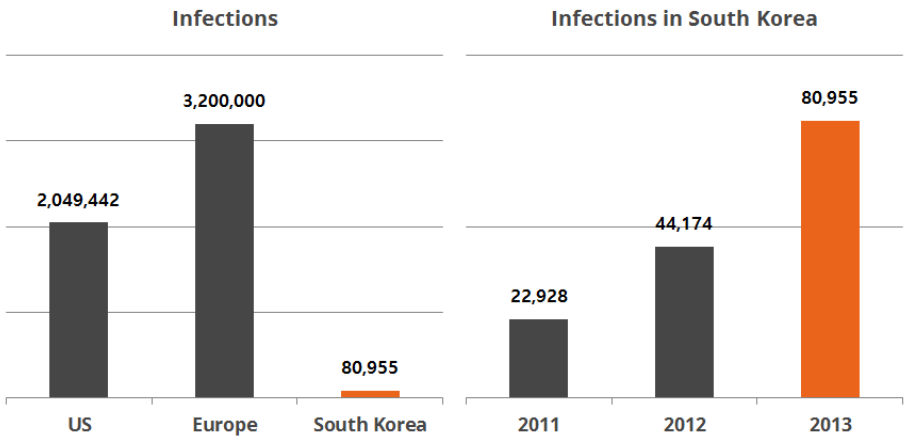


Figure 1.1. Morbidity of antibiotic resistance infections worldwide. The number of infections in South Korea has been dramatically increasing since 2011. (Antibiotic Resistance Threats in the United States, 2013, US CDC, European Antimicrobial Surveillance Network, 2013, ECDC, South Korea: Healthcare associated multidrug resistance report, 2014, Korea Centers for Disease Control & Prevention)

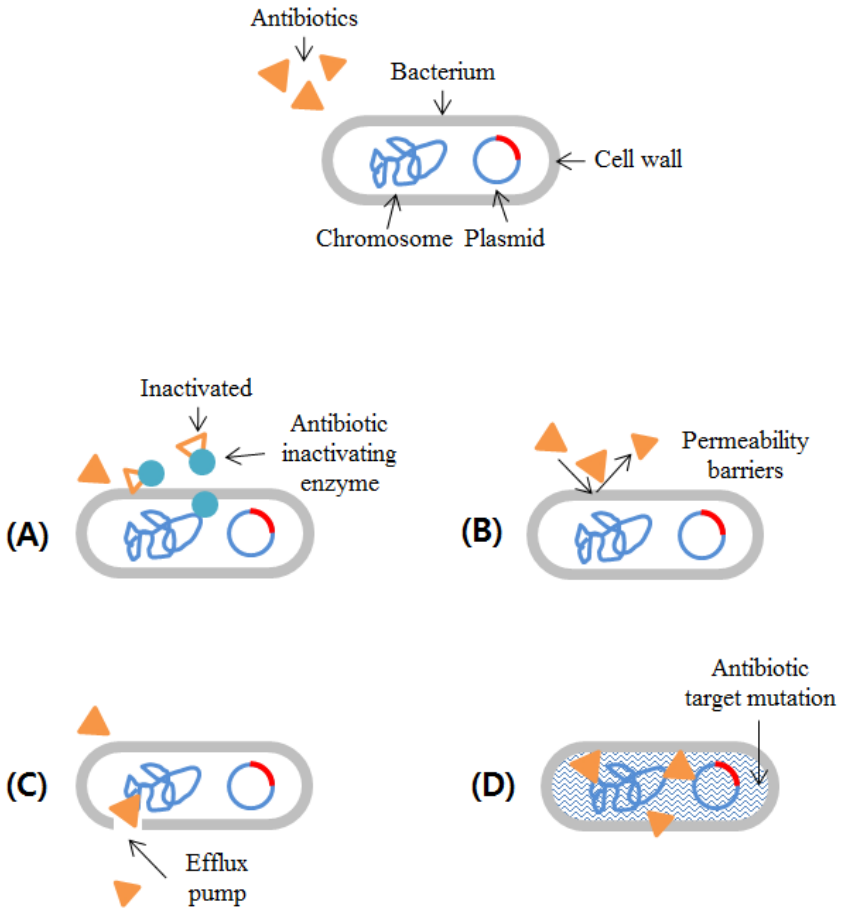


Figure 1.2. Antibiotic resistance mechanisms. Antibiotic resistant bacteria evade antibiotic actions through four common mechanisms. (A) Antibiotic inactivating or degrading enzymes are produced. (B) Permeability barriers prevent the antibiotic molecules from penetrating the membrane. (C) Efflux pump pumps out antibiotics that have entered the cell. (D) The target of the antibiotics mutates so the antibiotic fails to bind.

Table 1.1 Morbidity and mortality of antibiotic resistant infections in the United States (Antibiotic Resistance Threats in the United States, 2013, US CDC)

Infection	Annual cases (Estimated)	Death cases (Estimated)	Mortality rate (%)
Carbapenem-resistant Enterobacteriaceae (CRE)	9,300	610	6.56
Clostridium difficile Infections	250,000	14,000	5.60
Drug-resistant Neisseria gonorrhoeae	246,000	<5	N/A
Drug-resistant tuberculosis	1,042	50	4.80
Extended-spectrum β-lactamase producing Enterobacteriaceae (ESBLs)	26,000	1,700	6.54
Methicillin-resistant Staphylococcus aureus (MRSA)	80,000	11,000	13.75
Multidrug-resistant Acinetobacter	7,300	500	6.85
Streptococcus pneumoniae	1,200,000	7,000	0.58
Vancomycin-resistant Enterococcus (VRE)	20,000	1,300	6.50
Vancomycin-resistant Staphylococcus aureus (VRSA)	<5	<5	N/A

1.2 Objectives

The potential risk of antibiotic resistance is in case a particular bacterial strain had gained resistance to all antibiotics in the market, there will be no options to treat patients infected with these particular strains. Considering that most patients infected with antibiotic resistant bacteria acquired infections from hospitals, they are already low in level of immunity which makes physicians more difficult to give proper medical treatment. Mortality of some fatal antibiotic resistant bacterial infections in blood vessels ranges up to 50% which suggests that we are in need of an immediate action before we get to the last resort. While pharmaceutical companies risk the cost of developing new antibiotics (Christoffersen, 2006), the spread of bacteria evolved with new resistance genes may overtake the time-consuming process of developing new drugs from analysis, research, clinical trials, FDA approval to sales.

We will assume CRE(Carbapanem-resistant Enteriobacteriaceae) to be the bacterial agent in our study, also referred to as Super Bacteria in South Korea, due to the fact that these species are among the most fatal antibiotic resistant bacteria classified as "urgent", killing more than 9000 people every year and increasing more than ever before. The global spread of CRE is one of the most worrisome antibiotic associated infectious diseases since 2000 (Nordmann et al., 2011). CRE are gram-negative bacteria that inhabit the intestines of human which shows broad antibacterial power to carbapenems which includes E.coli and *K.pneumoniae* etc. In recent years, CRE strains with NDM-1 encoding genes were isolated from Europe, Americas, Africa and Asia (Nordmann et al., 2012; Poirel et al., 2011; Molton et al., 2013). Especially when looking into Asia, these resistance genes including NDM-1,

KPC-1, KPC-3, VIM-1 etc. were isolated from medical institutions in India, China and South Korea (Jeana and Hsueh, 2011; South Korea CDC, 2011). This implicitly means that South Korea is no more a safety zone for superbugs like CREs thus health authorities should prepare for potential risk of antibiotic resistance. As this public health issue is becoming progressively more difficult to deal with, we developed simulation models that have various advantages compared to traditional laboratorial approaches (Figure 1.3).

The goal of this study is to design simulation models of bacterial growth and antibiotic resistance through individual based modeling, and implement a framework for monitoring and predicting results when parameter values in the simulation model are adjusted. Further, we will compare simulated results with those of lab experiments under the same MIC (Minimum Inhibitory Concentration) values, thus as a final result, predict the effects of antibiotics when antibiotics are added to bacterial population with and without antibiotic resistance.

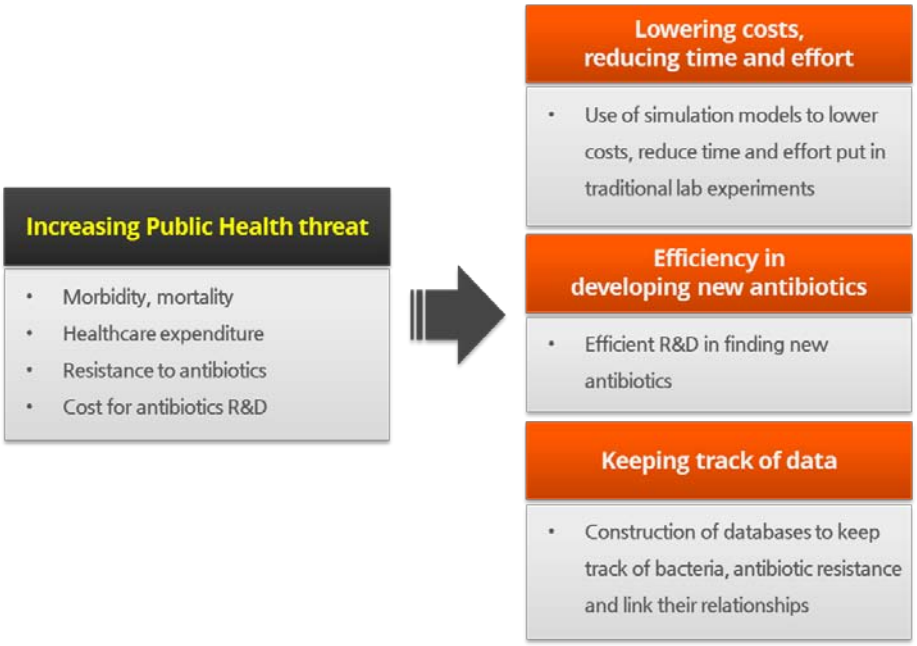


Figure 1.3. Advantages of computer simulation models in antibiotic resistance research. Using simulation models, we are able to lower costs, reduce time, gain efficiency and keep track of data when conducting research.

CHAPTER II.

MATERIALS AND METHODS

2.1 Modeling the bacterial population growth

Quantizing the population of bacteria is an extremely important process in bacterial growth simulation. The process is referred to as population dynamics, which monitors and predicts the quantity of cells in a bacterial population (Turchin, 2003; Onstad, 2003). Population dynamics is used in various fields of science and engineering including biology, ecology and demography, and we adapted the idea of population dynamics in our study to predict the number of bacteria in the simulation model.

2.1.1 Exponential and logistic models

Exponential growth and logistic growth models are mostly mentioned to explain the change of population in a group in terms of a macro perspective not from an individual perspective. Since all prokaryotes are known to divide cells as they grow, which is called prokaryote fission or binary fission, the most appropriate model that best describe bacterial growth would be the exponential model (Zwietering et al., 1990; Nykamp et al.). The exponential growth model can be explained as below.

If we assume that P_t and P_{t+1} are the number of bacterial cells at a given time t and t+1, the equation of P_t and P_{t+1} is as follows.

$$P_{t+1} - P_t = RP_t \quad \text{……...……(2.1)}$$

Since values for P_t and P_{t+1} can be filled in by results from lab experiments, we can find *R* to solve the number of bacteria P_t at a given time *t.*

$P_t = P_0R^t$, (R>1) ……...……(2.2)

In (2.2), P_0 is the initial number of bacteria, P_t is the number of bacteria at time *t*, and R^t is a constant that presents the rate or velocity of growth after time *t* has elapsed.

Depending on the temperature, pH, nutrients and more environmental factors, bacteria finds its best way to survive with its conditions. If conditions were fit, the growth of bacteria will be very much likely follow the curve of the exponential model. If however conditions are bad for survival, as nutrients become depleted and cells compete, the exponential model finds its limits on explaining further growth. That is to say, exponential models are suitable for explaining the initial period of bacterial growth, but it is insufficient to describe the latter part of growth.

In order to compensate the restraint on growth due to environmental factors, a constant limiting the maximum number M is added to the model, which is called the carrying capacity (Meyer and Ausubel, 1999). If we set the maximum estimated number of bacteria as M, we can rearrange P_t as follows.

P_t/M: Proportion of current population to maximum population …(2.3)

$1-(P_t/M)$: Amount left to reach the maximum population ……...…(2.4)

In the logistic growth model, growths inhibited by environmental limitations are compensated by multiplying variables to the exponential growth model (2.4).

$P_{t+1} – P_t = RP_t\ 1-(P_t/M)$……...……(2.5)

Using the models above we can describe the growth of bacteria,

quantify and compare the numbers with lab experiments and furthermore estimate the expected change in bacterial population as growth continues. Also, as the model sets the limit of population by adding the carrying capacity variable, we are able to effectively control the massive increase in computational costs during simulations. These kinds of models are examples of top-down approaches as introduced before, the best advantage is its simplicity in explaining all entities in a population as a whole, which is easily applicable in computer simulation. Despite the conciseness, this model lacks in explanation of the last part of the four phases of bacterial growth curve which is lag phase, exponential phase, stationary phase and death phase. Due to this defiance in explaining the death phase, it is unsuitable for our study to use these models since measuring the death of bacterial cells after the addition of antibiotics is the utmost important feature in simulations. Additionally, top-down models were not able to neither account for the individual aspects of the population nor explain molecular level interactions within the bacterial population or between bacteria and antibiotics. We therefore decided that we excluded the exponential and logistic growth models in our simulation and find a better option that would adequately represent the biological mechanisms and interactions that arise among bacteria and antibiotics.

2.1.2 Individual-based models

With models mentioned in Chapter 2.1.1, we were able to simply implement top-down models that describe the bacterial growth without environmental limitations. In order to design a more realistic model for the simulation, we are in need of a model that takes into account environmental factors and individual heterogeneities among bacterial population. In this study, we implemented the simulation models using IbM (Individual based Modeling) which is a greatly active method used in population dynamics.

Individual based modeling, also referred to as Agent-based modeling or Entity-based modeling is a modeling method where individual entities are

modeled and activities of these entities are tracked to observe how they affect the whole population (Bonabeau, 2002; Crooks and Heppenstall, 2012). This is contrary to how most models in population dynamics are unable to track individual entities and averages the value among entities within the population instead (SGPBI, 2002). Distinguishable advantages of IbMs for scientists are that IbMs are capable of developing a synergetic framework of conceptualizing a natural phenomenon, shaping up research methods, analyzing results and finally designing a model (Van Winkle et al., 1993).

IbM is basically consisted of two components. First component is the set of variables which can further be grouped into inner variables and outer variables. Inner variables are variables that represent the inner state of entities, in the case of this study, flags representing dead or alive, resistance to antibiotics, generation time, etc. Outer variables represent the environmental states that the entities belong to, in this case such variables like nutrient concentration, pH or acidity, temperature, diameter are included. Outer variables affect all entities within the population. The second component is the set of functions. These functions define actions of all entities whether they interact with each other or the environment. For example, we can consider how fast an antibiotic resistance gene transfers to others within a population, or the reaction rate of enzymes when antibiotics are added to the bacterial population.

Some limitations of population dynamics models mentioned in 2.1.1 can be resolved using IbM. By putting variables of survival states in each bacterium, it is possible to switch the status from alive to dead when the bacterium is unable to grow and divide, due to environmental conditions such as shortage in nutrients or insufficient geographical space. If each bacterium cannot meet the expected conditions, the survival flag will switch to ‘unable to grow’ or ‘dead’ in extreme circumstances. Aggregating all states of bacteria

in the population will give us insights of what is happening inside the population. Also, designing a model of nucleic acids of a bacterium will provide functions of what actions a bacterium should perform or what kind of protein a cell must encode when having a specific gene.

However, IbMs lack of standardized models compared to those of top-down approaches and focuses on individual actions rather than the whole population. Computational processes become heavier as number of entities increase which may build up as a major risk in simulation. Nevertheless, the inherent feature of IbM allowing modeling and simulations of individual entities was assessed to be a major advantage in our study.

In our study, we designed models of bacteria, antibiotics, enzymes using IbMs, and finally designed an environment model that includes all entities with specific conditions known to be suitable for bacterial growth. We implemented the growth of bacteria from molecular level, spread of enzymes, interactions between bacteria and antibiotics and other mechanisms to monitor and predict the consequences of adding antibiotics on specific conditions. Detailed information regarding the design and implementation of models will be dealt with in Chapter 2.3.

2.2 Modeling antibiotic resistance

The process of bacterial growth simulation and observing the effects of adding antibiotics is the most important part of this study. The more exact and confident with the results, the more chances you have in finding the proper antibiotics that overwhelms bacteria with antibiotic resistance. We will be able to efficiently predict the type and concentration of antibiotics that is best suitable in certain conditions through our simulation program.

As mention in Chapter 1, this study was aimed to simulate the growth and resistance of CRE (Carbapenem-resistant Enteriobacteriaceae). The reason was that not only CRE was one of the most dangerous infections in the United States (Prabaker and Weinstein, 2011; Braykov et al., 2013), but it was also designated recently as one of the six healthcare acquired antibiotic resistace infections in South Korea (Kim S. Y. et al., 2013; Kim M. N. et al., 2012), which means the South Korean government and healthcare institutions must take actions more aggressively on this public health issue. CRE is an Enterobacteriaceae with resistance to Carbapenems, such as E.coli and *K.pneumoniae*, meaning that non-resistant strains are easily isolated from the intestines of humans. Among all antibiotic resistant bacteria, we chose *K.pneumoniae* with resistance gene NDM-1 considering the urgency and fatalness the bacterial infection potentially have in the near future.

2.2.1 Resistance genes

Enteriobacteriaceae species are known to have genes that produce ESBL (Extended Spectrum Beta-lactamase), enzymes that hydrolyze beta-lactam antibiotics. These species have the potential to become major risks in public health since 10-40% of E.colis and *K.pneumoniae*s have the ability to code ESBLs (Rupp and Fey, 2004). Interestingly, however, this did not

happen to be a crucial issue in public health since ESBL enzymes are treatable by Carbapanems and resistance against these antibiotics are remarkably low compared to resistance against beta-lactam antibiotics.

After isolation of KPC(*Klebsiella pneumoniae* carbapenemase) gene from a *K.pneumoniae* strain from North Carolina, US (Yigit et al., 2001), a set of genes that encodes enzymes that reacts with Carbapenems and hydrolyzes them, reports of this fatal Carbapenem-resistant strain have been increasing over the years. More genes were found other than KPCs, such as NDM-1 which was first isolated from India in 2008 and have spread rapidly not only to adjacent countries like Pakistan, but also to geographically distant countries like the United Kingdoms, United States and Japan. NDM-1 producing *K.pneumoniae* was first isolated even in South Korea in 2010. More carbapenemase encoding genes such as KPC, NDM, IMP, VIM etc. are organized in Table 2.1.

In this study, a database of carbapenemase encoding genes was constructed. Basic information including gene names and sequences were retrieved from the NCBI website. The purpose of collecting genes is if a bacterium has a carbapenemase encoding gene, carbapenemase would be produced from the bacterium when antibiotics are added.

2.2.2 Horizontal gene transfer

There are three ways how bacteria acquire genes from the external environment, conjugation, transduction and transformation. Conjugation is when a recipient cell receives genetic material from a donor cell through the conjugative pili or the sex pili with direct contact. In this case, a donor cell which has the resistance gene contacts a donor cell when they are physically close enough to connect to each other, and gives out the gene through the pili.

Most antibiotic resistant bacteria are known to acquire genes through conjugation. Transduction is when genetic material is transferred from one to another not through direct contact but through a virus called bacteriophage. A bacteriophage acquires genes from bacteria in its own genome and carries the genetic information to other bacteria. The last method is transformation where genetic material is acquired from external environment by DNA recombinant technology and incorporated directly into the recipient cell and also includes acquiring genes from dead cells.

Looking into Table 2.1 shows that most carbapenemase encoding genes are located inside the plasmid not in the chromosome of a bacterium. CRE species such as E.coli and *K.pneumoniae* acquires antibiotic resistance by transferring resistance genes to and from plasmids. Especially, KPC-3 (Klebsiella Pneuomoniae Carbapenemase-3), a gene that encodes an enzyme originally produced from *K.pneumoniae* were isolated from E.coli which means that gene transfer among bacteria occurs not only between the same species but also between different species (Goren et al., 2010). This implies the spread of antibiotic resistance may be faster than we think since they are transferable to other species as well.

In our simulation model, we defined rules for gene transfer based on the assumption that horizontal gene transfer is the same as the mechanism of conjugation through bacterial plasmids (Gregory et al., 2008). Three gene transfer mechanisms were implemented including one vertical, two horizontal gene transferring mechanisms (Figure 2.1). According to previous studies, recipient bacteria were not able to acquire genes by conjugation when the size of the cell was 60-70% the size of a full grown. When cell sizes exceeded 80% of the size of a full grown normal cell, approximately 75% of horizontal gene transfers through conjugation occurred (Seoane et al., 2011). Therefore, we made a first rule to allow gene transfers through plasmids only when the recipient cell reached more than 80% of its original full grown size.

The second rule for horizontal gene transfer is setting a parameter that controls the frequency of gene transfer occurrence between bacteria. This parameter, also referred to as transfer frequency or conjugation rate, defines the successful transfer rate of acquiring genes through plasmids (Seoane et al., 2010). The bacterium that gives out its genes is called the donor cell (D), the bacterium that is capable of receiving the genes are called the recipient cell (R) and the bacterium that already have received the genes is called the transconjugant cell (T). Using these three kinds of cells, we can assume the frequency of transfers by the following equation.

$$\text{Transfer frequency} = T * D^{-1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.6)$$

For the simulation in this study, we set the Transfer frequency to $(1.3 \pm 0.5) * 10^{-4}$ based on an experiment conducted with carbapenem resistant *K.pneumoniae* strains possessing NDM-1 genes (Potron et al., 2011).

2.2.3 Antibiotics-bacteria interactions

Bacteria that have acquired antibiotic resistance genes are capable of fighting against antibiotics with various mechanisms when they are exposed to antibiotics. The mechanism in CRE starts from the bacteria encoding an antibiotic hydrolyzing enzyme called carbapenemase (Weinstein et al., 2011). While their action mechanisms are similar, these carbapenemases are classified into three groups by their molecular form, class A, class B and class C (Queenan and Bush, 2007). We decided to simplify the interaction by assuming that all carbapenemases are just one group since there were limitations in implementing different mechanisms of all three classes.

The Michaelis-Menten kinetics equation was used to quantify and calculate the rate of how fast a carbapenemase hydrolyzes carbapenem molecules. Using the equation, we set the reaction rate (mM/sec) of enzymes

and substrates, in this case carbapenems. Putting the concentration of substrate as [S] and the Michaelis constant as K_m, the following equation for the reaction rate V_0 can be solved.

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]} \quad \text{……(2.7)}$$

Also, catalytic conversion efficiency or the turnover rate which is the amount of substrate molecules transformed to products shown as $k_{cat} = V_{max}/[E]_t$, can be substituted in equation (2.7) as shown in the following equation. $[E]_t$ is the concentration of enzymes at a given time *t*.

$$V_0 = \frac{k_{cat}\,[E]_t\,[S]}{K_m + [S]} \quad \text{……(2.8)}$$

In this study, we calculated the V_0 based on the equation above and applied it to the simulation model. Values for k_{cat}, K_m were set based on previous experiments of carbapenem resistant NDM-1 producing *K.pneumoniae* and E.coli (Kim et al., 2011; Shen et al., 2013). When antibiotics are added to the bacterial population, bacteria without resistance will be inhibited from division due to the interference in their cell wall synthesis. On the other hand, cells with resistance will encode and produce antibiotic hydrolyzing enzymes at a rate of V_0 from (2.8) and lower the concentration of antibiotics.

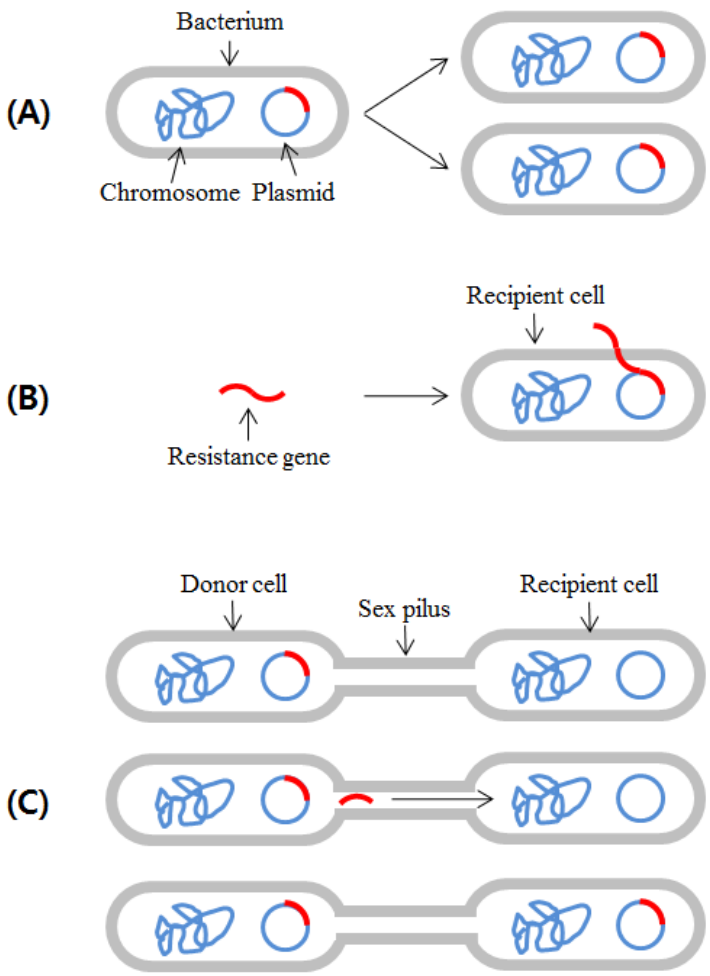


Figure 2.1. Gene transfer mechanisms implemented in this study. Three gene transfer mechanisms were implemented in the simulation model: (A) Vertical gene transfer during binary fission, (B) transformation and (C) conjugation.

Table 2.1. Examples of carbapenemase encoding genes

Type	Description	Gene	Location	Size(bp)
IMI-1	Class A Carbapenemase IMI-1	bla_{IMI-1}	Chromosome	2,572
SFC-1	Class A Carbapenemase SFC-1	bla_{SFC-1}	Chromosome	1,140
KPC-2	Class A Carbapenemase KPC-2	bla_{KPC-2}	Plasmid	1,146
NDM-1	Class B New Delhi Metallo-beta-lactamase	bla_{NDM-1}	Plasmid	1,057
OXA-48	Class D Carbapenemase OXA-48	bla_{OXA-48}	Plasmid	1,038
VIM-1	Class B Metallo-beta-lactamase VIM-1	bla_{VIM-1}	Plasmid	1,041
IMP-4	Class B Metallo-beta-lactamase IMP-4	bla_{IMP-4}	Plasmid	963

2.3 Modeling the environment

In order to provide flexibility in simulations, most environmental factors were set by parameters instead of fixed values. Users of the simulation program can first set the parameters before simulation, such as the diameter type of the virtual plate, initial concentration of nutrients, temperature and acidity. In this study, we considered the medium where bacteria grow as an agar plate with a diameter of due to constraints to fit the size of the monitor screen. The temperature was initially set to 37˚C, referring to literature that the best temperature for *K.pneumoniae* was between 35-45, E.coli was 37 (Paterson et al., 2004). Acidity was set to pH 6.0, however we could not find causality between this number and the outcomes and due to weak evidence in literatures, we excluded acidity from user parameters and fixed the number to 6.0. All factors mentioned above were saved and loaded in files using the standard JSON format (Crawford, 2006) (Figure 2.2). Parameters for bacteria and antibiotics factors were also saved in this parameter file.

```
{
   "environment":{
      "diameter":60,
      "culture medium":"agar plate",
      "nutrient level":2000,
      "nutrient diffusion coefficient":0.2,
      "temperature":37.0,
      "pH":6.0,
      "max bacteria":3000,
      "max enzymes":20000
   },
   "bacteria":{
      "name":"Klebsiella pneumoniae(K. pneumoniae)",
      "gram type":"gram-negative",
      "known resistance":[
         "carbapenem"
      ],
      "producing enzyme":[
         "carbapenemase"
      ],
      "minimum diameter":0.3,
      "maximum diameter":1.0,
      "generation time":40,
      "mobility":"no"
   },
   "antibiotics":{
      "name":"imipenem",
      "type":"carbapenem",
      "diameter":0.6,
      "action mechanism":"inhibition of cell wall synthesis",
      "antibiotics diffusion coefficient":0.2,
      "concentration":100.0
   }
}
```


Figure 2.2. An example of parameters for the simulation program in JSON format. Parameters for bacteria, antibiotics and the environment are loaded and saved as files. Files are saved in JSON format to enhance readability and provide interoperability among users.

2.4 Modeling the molecular movements

2.4.1 Data structure of molecules

Data structures of all entities composing the simulation model were designed. Antibiotic molecules and enzymes were implemented as vector arrays to avoid unnecessary computation. However, it was impossible to implement bacterial data into arrays due to its complexness. Therefore we borrowed a Quadtree data structure and modified it to be suitable for our application.

Assuming that bacteria were placed in a virtual plate, computational cost becomes an extremely important issue as bacteria pass the binary fission process. As newly divided bacterial cells grow and possess space, we have to consider how the simulator will react when no more space is allowed to divide or efficient algorithms to finding which direction or course will bacteria tend to divide or grow. For this reason, we implemented a Quadtree data structure which is similar to various tree data structures such as B-trees, B+trees and Octrees (Meagher, 1982; Samet et al., 1984) (Figure 2.3). Quadtree starts with equally dividing a space into four spaces. Data are stored in these four spaces depending on their geographical position. As data increase and reaches the maximum limit allocated to the space, it is divided again into four spaces, and this process occur recursively until it reaches the maximum depth. This result in a substantially efficient computation when detecting collision between bacteria compared to arrays where an iterator browses the whole data structure for the same task. This is especially useful for spatial repositioning of bacteria or transferring genes between adjacent bacteria.

Data structure for antibiotics were implemented using an array with a size of a n*n matrix. n in this matrix was the pixel size of the width or height of the virtual plate, divided by 100 for computational efficiency. Instances

stored in this array have information of the type and concentration of antibiotics (Figure 2.4).

2.4.2 Molecular movements

Considering the division, diffusion and crowding mechanisms of bacterial cells, efficient methods were needed to properly implement the nature of bacteria and antibiotics for the simulation model, particularly at the molecular level. All molecules including bacterial cells, enzymes, antibiotic molecules, nutrients were taken into account. Modeling the movements of bacteria and molecules were required to implement the nutrient intake and binary fission of bacteria and interactions between bacteria, antibiotics and enzymes.

Bacteria were designed to divide to an adjacent area with the highest concentration of nutrients instead of any random direction. There are eight directions where bacteria can divide into and in rare cases when two or more directions have the exact same amount of nutrients, only then bacteria were allowed to divide into random directions (Figure 2.5). Also, when bacteria shared the same space or when they are exactly overlapped, we considered this as a collision between cells and applied actions to all the bacteria involved so that they push each other away from the amount proportional to their molecular mass (Figure 2.6). For example, if the molecular mass of a bacterial cell was twice larger the amount of another cell that had collided, the colliding cell will be pushed away by twice the amount of the movement of the original cell.

For nutrient molecules, as nutrient intake gradually increases in particular areas of the virtual plate, the concentration of nutrients decrease. This causes difference in concentration inside the plate which leads to

diffusion. We implemented a diffusion mechanism using the discretized version of Fick's first law of diffusion (Ginovart et al., 2002)(Figure 2.5) for the diffusion of nutrients and antibiotics. While this coefficient varies depending on the type of medium where bacteria grow, it was difficult to find evidence in previous studies of the changes in outcomes when changing values of this coefficient. Therefore, the diffusion coefficient used in the algorithm was mostly fixed to 0.5 on a 0 to 1.0 scale.

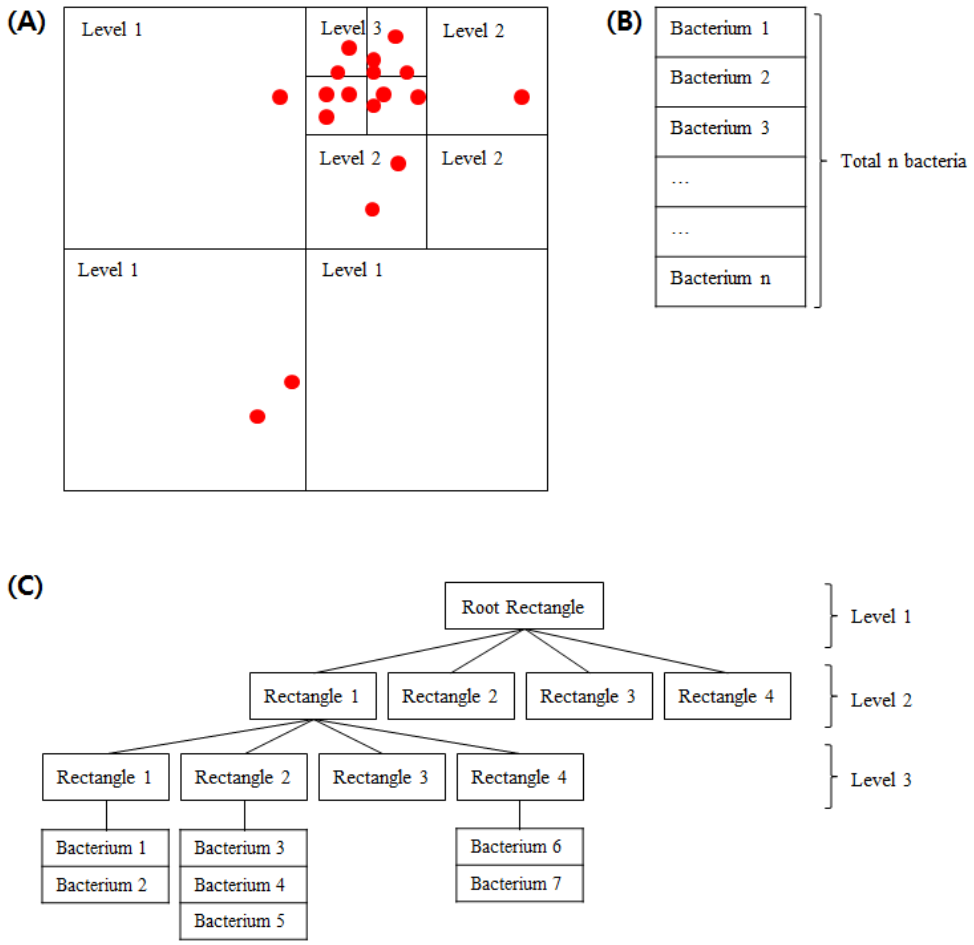


Figure 2.3. Data structure of bacteria. (A) represents how the Quadtree data structure divides a rectangle space into four identical rectangles objects are being added. The diagram shows if more than 10 objects are within the same rectangle, then the rectangle is divided. (B) holds all bacteria objects in memory. (C) shows the data structure of a quadtree with three levels, same as (A) above.

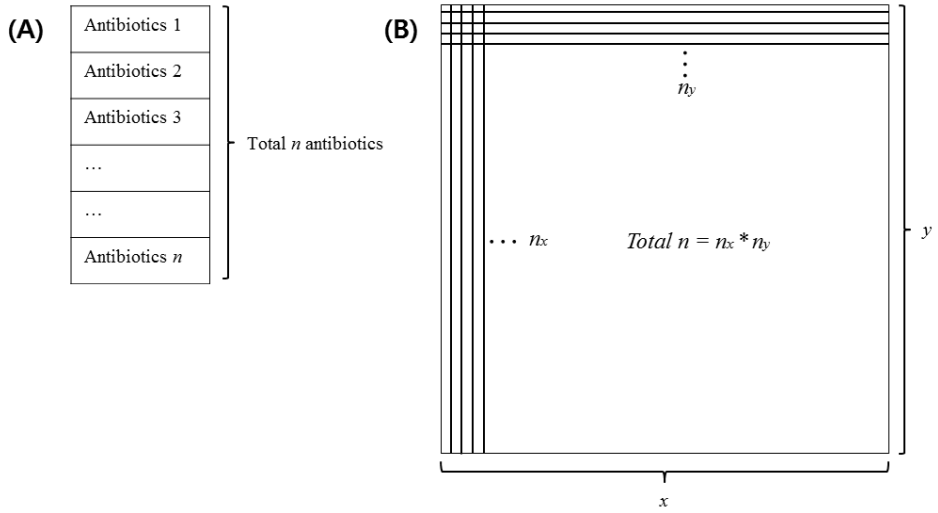


Figure 2.4. Data structure of antibiotics. (A) is a vector array in memory which holds all antibiotic objects. (B) depicts the grid of antibiotics in the plate.

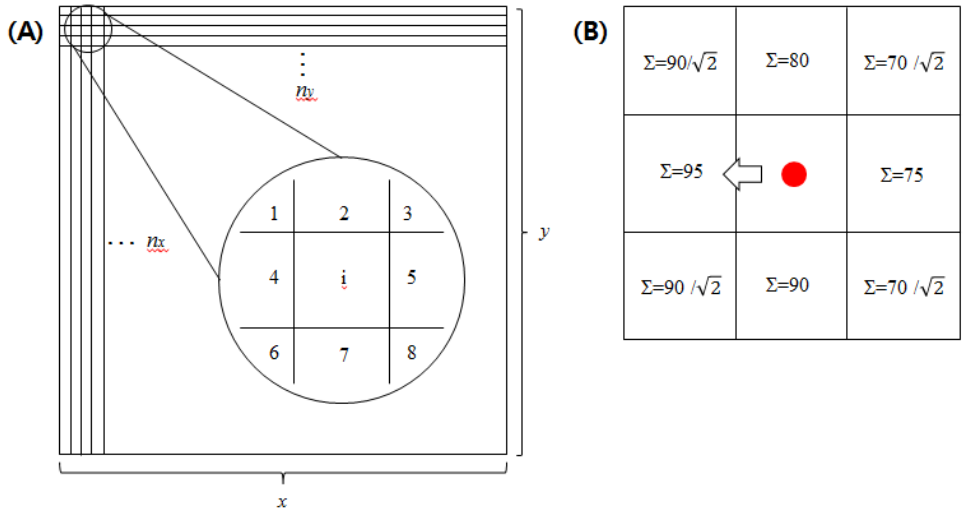


Figure 2.5. Diffusion of nutrients and bacterial cell movements. (A) The first step of nutrient diffusion is performed by calculating the difference between “i" and all surrounding areas from 1 to 8. This step goes on a loop for the whole area until “i" becomes greater than or equal to (n_x * n_y). (B) After nutrient diffusion, bacterial cells are later divided towards the direction with the highest nutrient level.

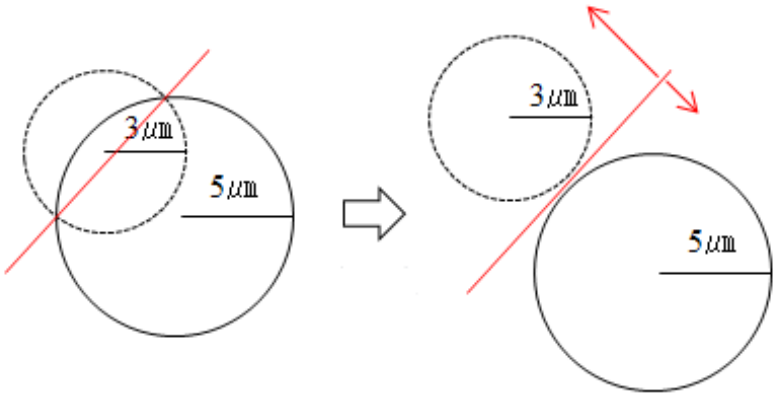


Figure 2.6. Collision of bacterial cells. When cells collapse or collide, if both the same size, we assume they have the same molecular weight, therefore force them to push the same amount opposite to each other. If cells are different in size, the amount of cells pushing each other away is proportional to the diameter of the cells.

2.5 Database construction

Databases were constructed in order to store data that are needed for simulations, and to further expand the simulation model to a variety of other bacterial species and antibiotics. Three tables were implemented that stores data of resistance genes, genomes with resistant genes, and data of experimental results which holds antibiotic concentrations against specific species of bacteria (Figure 2.7).

The genes table was designed to store information of antibiotic resistance genes. The columns include the name of the resistance gene, description, accession identifier which points to the NCBI website, organism of where the gene isolated from, accession identifier for the organism, gene size in base pairs, whether the gene is virulent or resistant, its FASTA file path in the local server, and its nucleotide sequence. This table was intended to be used when the user inputs genetic sequences into the ARSim program upon conducting simulations. Although this feature was not fully implemented at this stage of study, data were gathered in order to provide information of virulence and resistance of a particular gene (Table 2.2). The genomes table was similarly designed to store information of microorganisms that are known to have antibiotic resistance. The columns of this table are the type of the bacteria, name of the isolated strain, accession identifier link to NCBI, number of contigs, length of sequence in base pairs, general description, the FASTA file path, and the nucleotide sequence. Like the preceding genes table, the genomes table was not yet used by the simulation in this study but provides meaningful information of genomes that are associated with antibiotic resistant bacteria (Table 2.3). All raw data that have been organized into these tables were acquired from the NCBI website. Lastly, the MIC table was designed for use during simulations when the bacteria queries for the information of antibiotics for molecular interactions. The table

consists of four columns, antibiotics, bacteria, resistance name, and the reported MIC value from previous laboratory experiments (Table 2.4). During simulations, when bacteria are exposed to antibiotics, the bacterial cells constantly compare the antibiotic concentration of their patch with the MIC in the database to make further actions.

Although this study is more focused on running simulations on designed models, the quality of databases is the key to the preciseness of the simulation. Therefore, we constructed a database that can be potentially used with the simulation models in this study, or even independently by providing useful information of the genetic factors of antibiotic resistant bacteria.

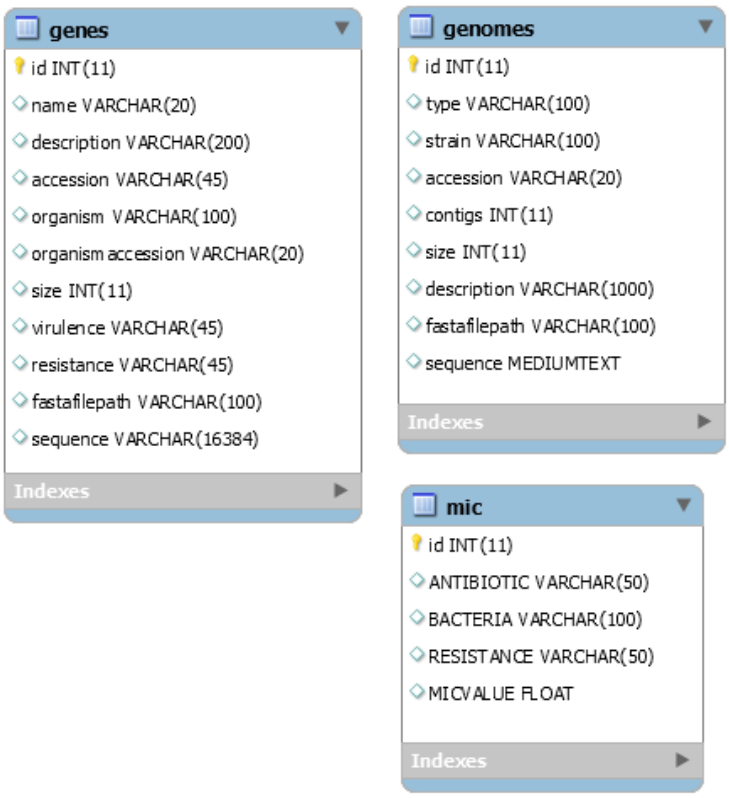


Figure 2.7. A diagram of the constructed database. Three tables were implemented for use in the ARSim program.

Table 2.2. Example of entries in the Genes table.

DB Table: Genes				
Columns	Entry1	Entry2	Entry3	Entry4
id	1	2	3	4
name	blaNDM-1	blaNDM-1	blaNDM-1	D647_p5006
description	New Delhi metallo-beta-lactamase	metallo-beta-lactamase (MBL) NDM-1	metallo-beta-lactamase NDM-1	carbapenem-hydrolyzing beta-lactamase KPC-3
accession	18983573	13914125	18983798	13914487
organism	*Klebsiella pneumoniae* KP1	*Klebsiella pneumoniae*	Enterobacter cloacae	*Klebsiella pneumoniae* (strain: ST258)
organism-accession	NC_023908.1	NC_019163.1	NC_023914.1	NC_019155.1
size	1191	1057	1057	1146
virulence	N	N	N	N
resistance	NDM-1	NDM-1	NDM-1	KPC-3
fastafilepath	18983573.fasta	13914125.fasta	18983798.fasta	13914487.fasta
sequence	TTGAATTCGCCC…	ATGGAATTGCCC…	ATGGAATTGCCC…	ATGTCACTGTAT…

Table 2.3. Example of entries in the Genomes table

DB Table: Genomes				
Columns	Entry1	Entry2	Entry3	Entry4
id	1	2	3	4
type	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	Klebsiella pneumonia
strain	ST15 NDM-1	BIDMC 24	CCBH13327	BWH 2
accession	CDQG00000000	AYHY00000000	JSER00000000	JCNQ00000000
contigs	98	20	106	24
size	5987607	5381634	6023590	5583740
description	*Klebsiella pneumoniae* strain ST15 NDM-1, whole genome shotgun	*Klebsiella pneumoniae* BIDMC 24, whole genome shotgun sequencing	*Klebsiella pneumoniae* strain CCBH13327, whole genome shotgun. Draft genome sequences of three NDM-1 producing Enterobacteriaceae	*Klebsiella pneumoniae* BWH 2, whole genome shotgun sequencing
fastafilepath	CDQG01.1.fsa_nt	AYHY01.1.fsa_nt	JSER01.1.fsa_nt	JCNQ01.1.fsa_nt
sequence	…	…	…	…

Table 2.4. Example of entries in the MIC table

DB Table: MIC					
Columns	id	antibiotic	bacteria	resistance	micvalue
Entry1	1	Imipenem	*Klebsiella pneumoniae*	NDM-1	32
Entry2	2	Imipenem	*Klebsiella pneumoniae*		0.094
Entry3	3	Meropenem	*Klebsiella pneumoniae*	NDM-1	32
Entry4	4	Meropenem	*Klebsiella pneumoniae*		0.064
Entry5	5	Aztreonam	*Klebsiella pneumoniae*	NDM-1	256
Entry6	6	Aztreonam	*Klebsiella pneumoniae*		0.094

2.6 Implementing the simulation program

To run simulations of designed models of bacteria, antibiotics, enzymes and their interactions, we developed a simulation tool (Figure 2.8). The first step to developing the program was to design simulation models based on individual based modeling. As mentioned above, we designed four models that make up the process of antibiotic resistance, bacteria, antibiotics, enzymes and the environment (Figure 2.9). Parameters for bacteria include the initial and maximum diameter of the cell, coordinates on the virtual plate, generation time, metabolic rate, etc., and the maximum population of bacterial cells created is limited to control the performance of the tool. Parameters for enzymes include the diameter, half-life and the reaction rate against substrates. For antibiotics, type of mechanisms of action was set as a parameter to control reactions with the enzyme. Also diffusion of antibiotics depending on the difference of concentrations was implemented similar to the diffusion of nutrients. All functions and variables mentioned above are implemented as classes through an objected oriented approach (Figure 2.11, Figure 2.12, Figure 2.13, Figure 2.14, Figure 2.15).

The first procedure of the simulation starts with initializing the environmental parameters and growing bacteria (Figure 2.10). Users are able to decide when to insert antibiotic resistance genes into bacteria which cause mutation. As the population grows to form a colony, antibiotics are added to observe and predict the effects. Precisely, parameters are set for all entities in the simulation model and the moment when a user clicks the start button is when the simulation actually starts. Then at any given time, the user grows antibiotic resistant bacteria by putting resistance genes into the virtual plate. Finally the user decides which antibiotic with a certain level of concentration to be added to the population which is the last procedure of the simulation process.

The name of the simulation tool was named ARSim, which stands for Antibiotic-Resistance Simulator. The program was made with Java and was

tested on Linux and Windows platforms. Considering that the target users for this program are scientists or researchers in clinical fields that are not familiar to in-depth computer operations, this program was built so it could be run on typical PCs or laptops rather than UNIX based workstations or clusters(Tang and Wang, 2009).

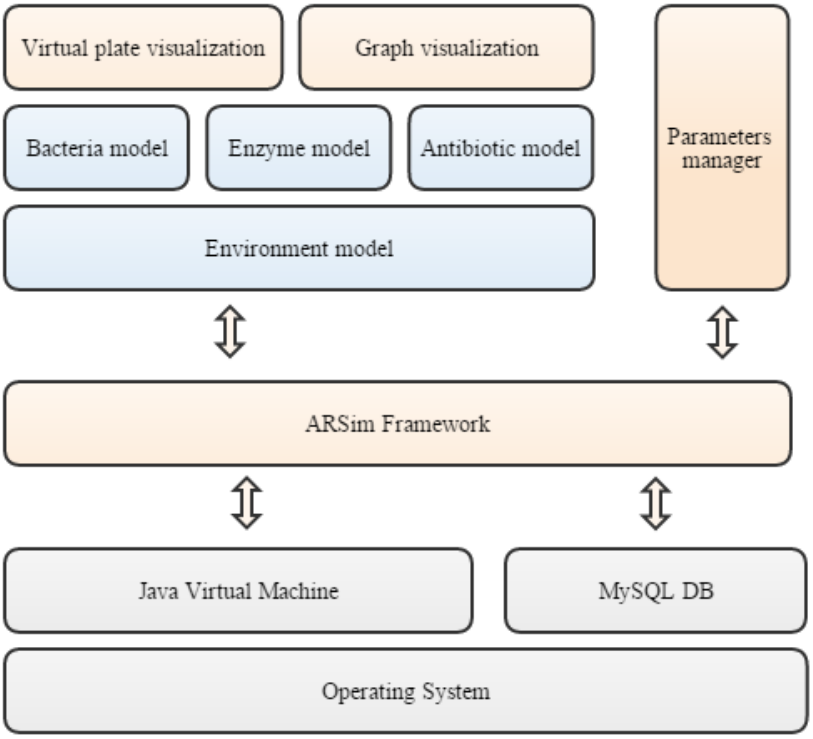


Figure 2.8. Stack of the simulation program ARSim. The program is developed on top of the JVM and uses MySQL as the local database.

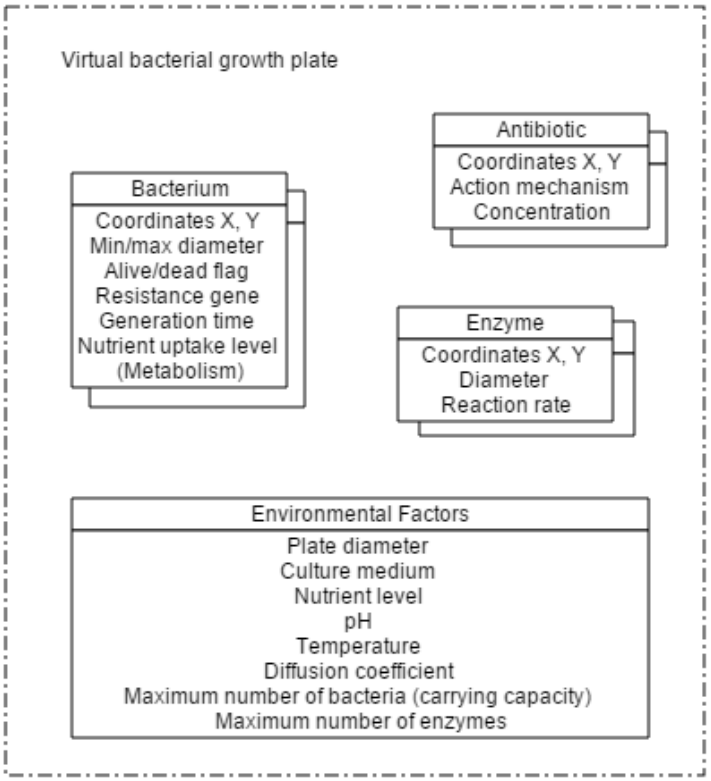


Figure 2.9. Diagram of models designed for the simulation program. The simulation model is consisted of four major entities: bacteria, antibiotics, enzymes and the environment.

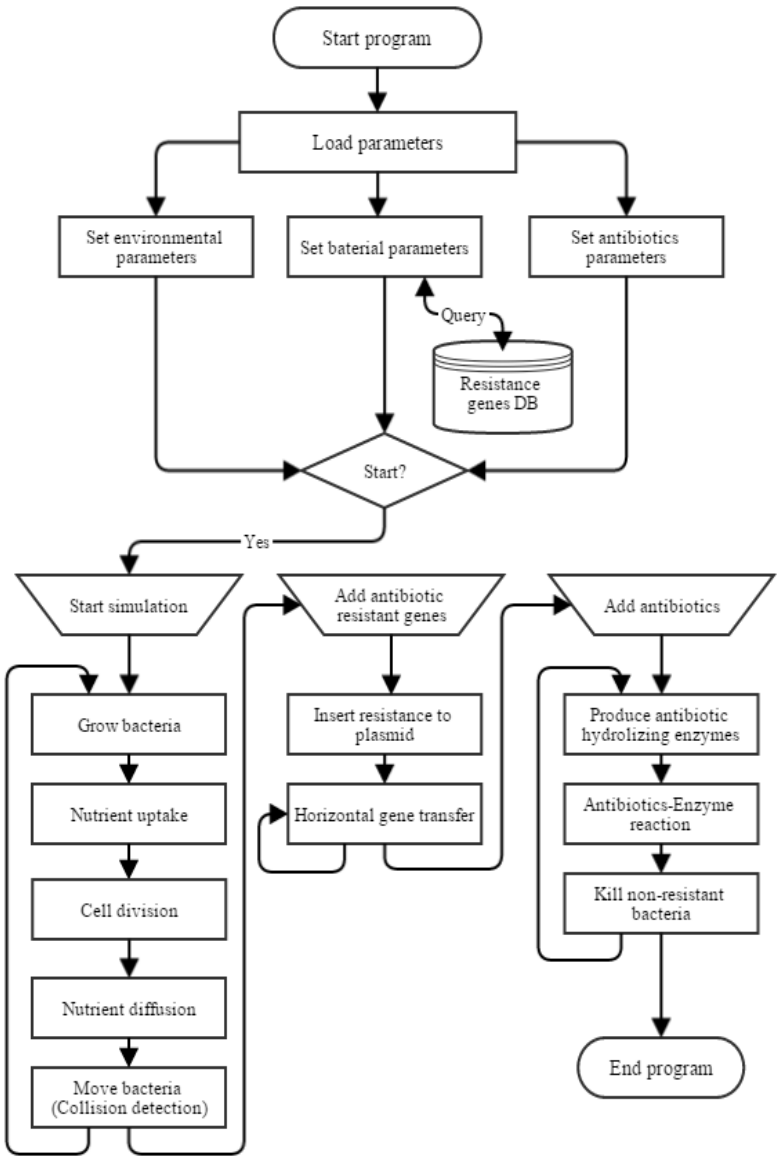


Figure 2.10 Flowchart diagram of the simulation program. Simulation begins with loading parameters and initializing bacterial growth in a virtual patch plate. During or after bacterial colonization, users decide when to add antibiotic resistance genes into the colony. Simulation ends by determining changes when antibiotics are applied to the plate.

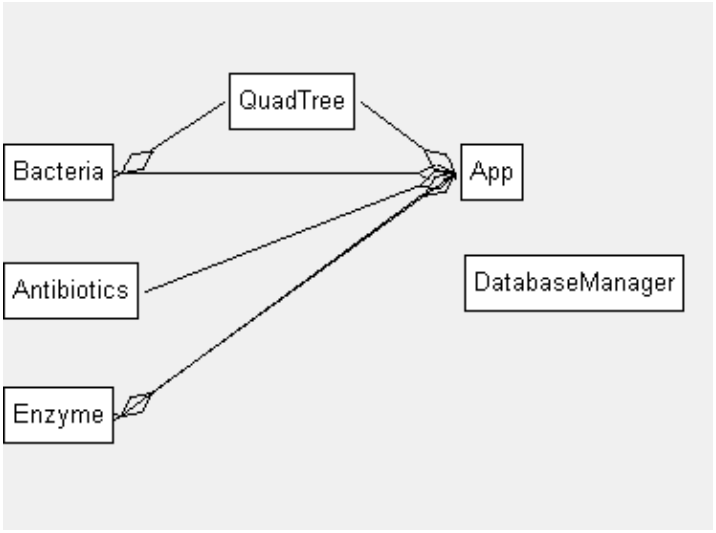


Figure 2.11. A basic class diagram of the simulation program. The simulation program is consisted of six classes: the main application class, bacteria class, quadtree class, antibiotic class, enzyme class and the database manager class.

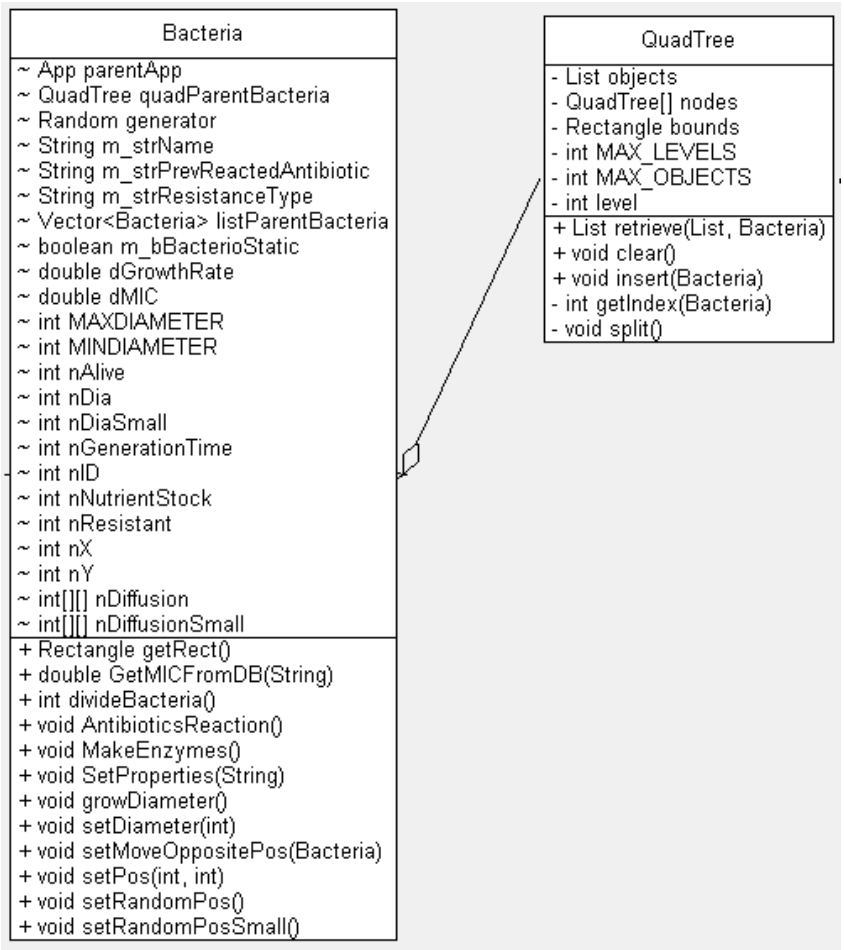


Figure 2.12. A class diagram of the Bacteria and Quadtree class

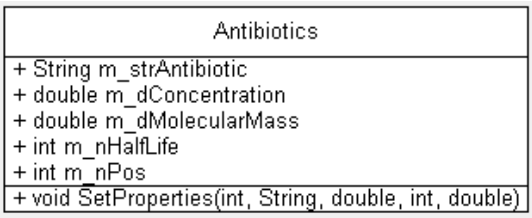


Figure 2.13. A class diagram of the Antibiotics class

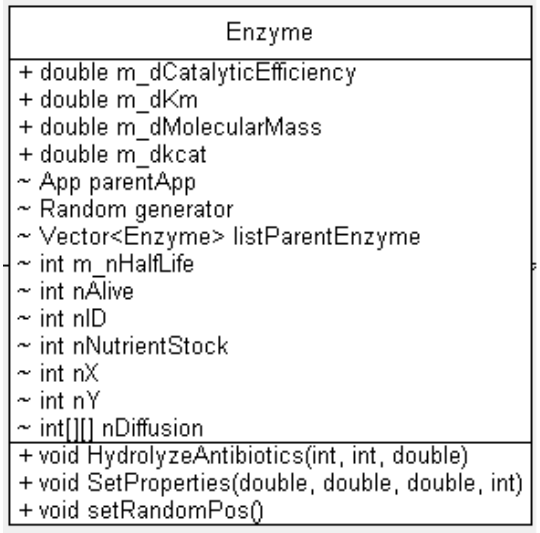


Figure 2.14. A class diagram of the Enzyme class

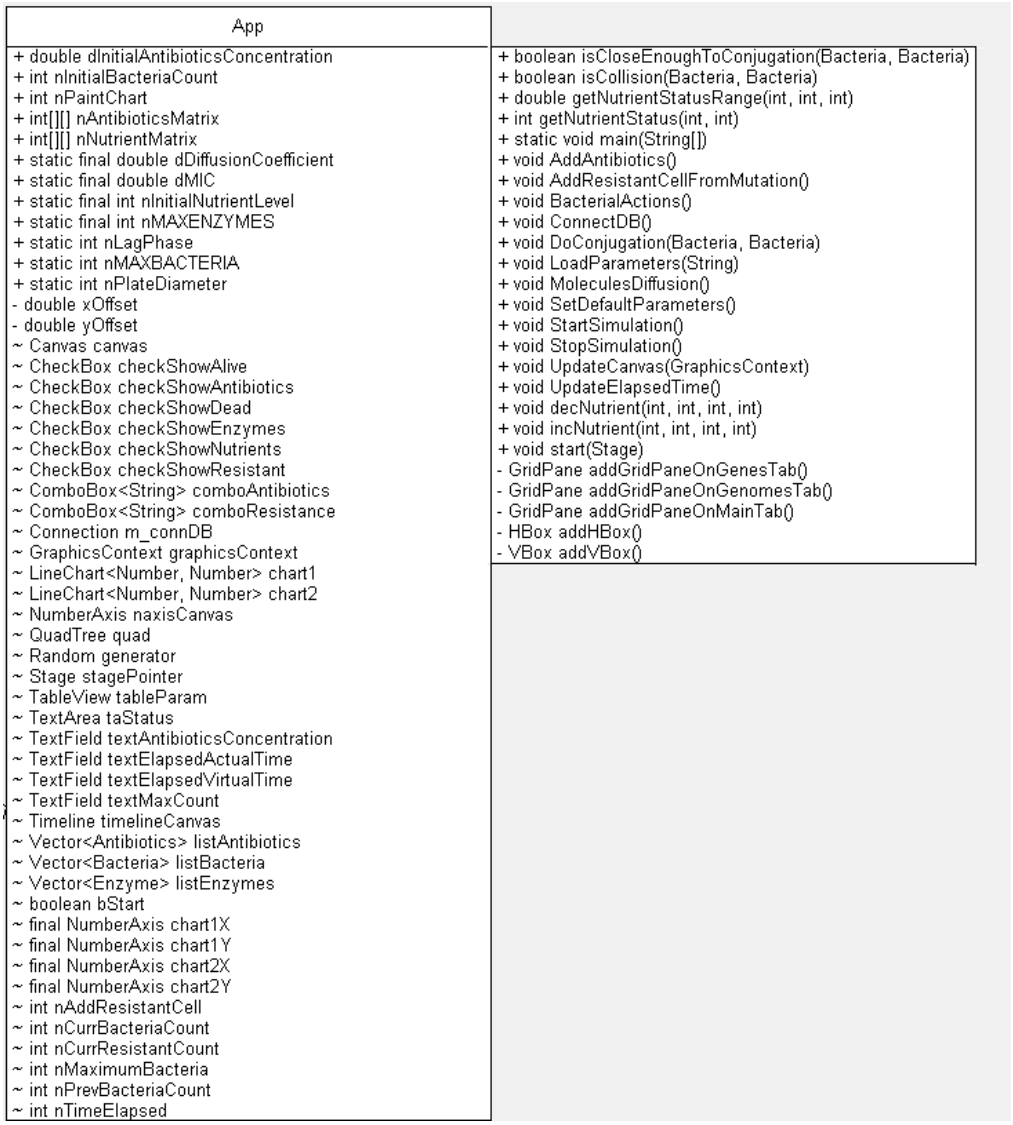


Figure 2.15. A class diagram of the App (main application) class

CHAPTER III.

RESULTS

We conducted various experiments of simulation models using the developed ARSim program (Figure 3.1). Simulations were classified into two groups of experiments. The first set of simulations was implementing and observing the growth of bacteria and the second set was predicting the consequences of antibiotics addition to multiple groups of bacteria.

3.1 Simulation of the bacterial growth model

The initial step of the antibiotic resistance simulation was to run the bacterial growth simulation model. We ran simulations based on the designed model and compared the results with the four phases of bacterial growth.

First, we put a few bacteria on the virtual plate and set the parameters for the model. Bacterial species were set to *K.pneumoniae*, minimum diameter was set to 0.3μm, maximum diameter was set to 1.0μm and the generation time was set to 40 minutes. For the environmental parameters, nutrient level for 1.0*1.0μm^2 was set to 300, diffusion coefficient was set to 0.5, lag phase to 60 and the nutrients emitted from dead cells were set to 10. Parameters mentioned for this simulation are listed in Table 3.1.

The result of the bacterial growth simulation was as shown in Figure 3.3 and Figure 3.4. During the experiment, changes in population by time were shown as a graph (Figure 3.3) and the changes were visualized to be able to observe the phenomenon more intuitively (Figure 3.4). ARSim was

developed to be capable of showing this information directly inside the program or exporting the information to files with raw data and screenshots (Figure 3.2). The results showed that changes in the population of bacteria were not exactly the same but arguably similar to the four phases of the typical bacterial growth curve. After the adaptation to the environment for approximately 3 hours, the bacterial population entered into a deep exponential curve for 6 hours. Following was the stationary phase when the number of dead cells and newly divided cells meet equilibrium and later the population entered the death phase as the number of cells gradually decreased. In Figure 3.4, red colored cells were represented as live cells, yellow as dead cells. The default background color was black when the nutrient level was the full amount of the nutrient level set through parameters, and gradually brightened to white as the concentration of nutrients decreases. The whole process of observing changes of the bacterial cells was continued for 18 virtual hours in the simulation and approximately 3 minutes in real world.

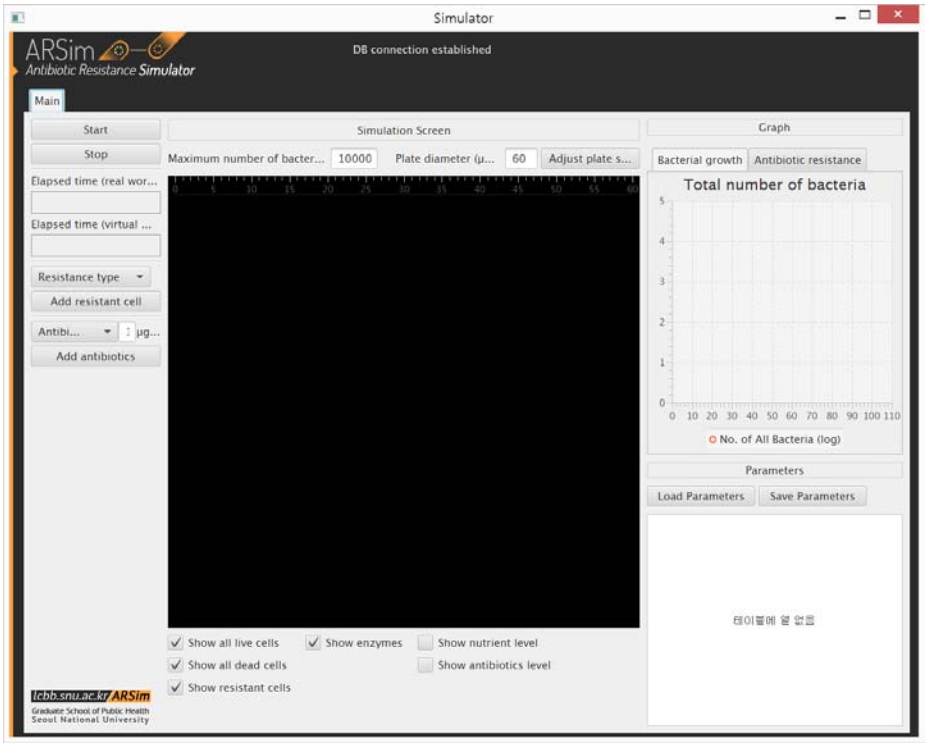


Figure 3.1. Screenshot of the simulation program ARSim for bacterial growth and antibiotic resistance.

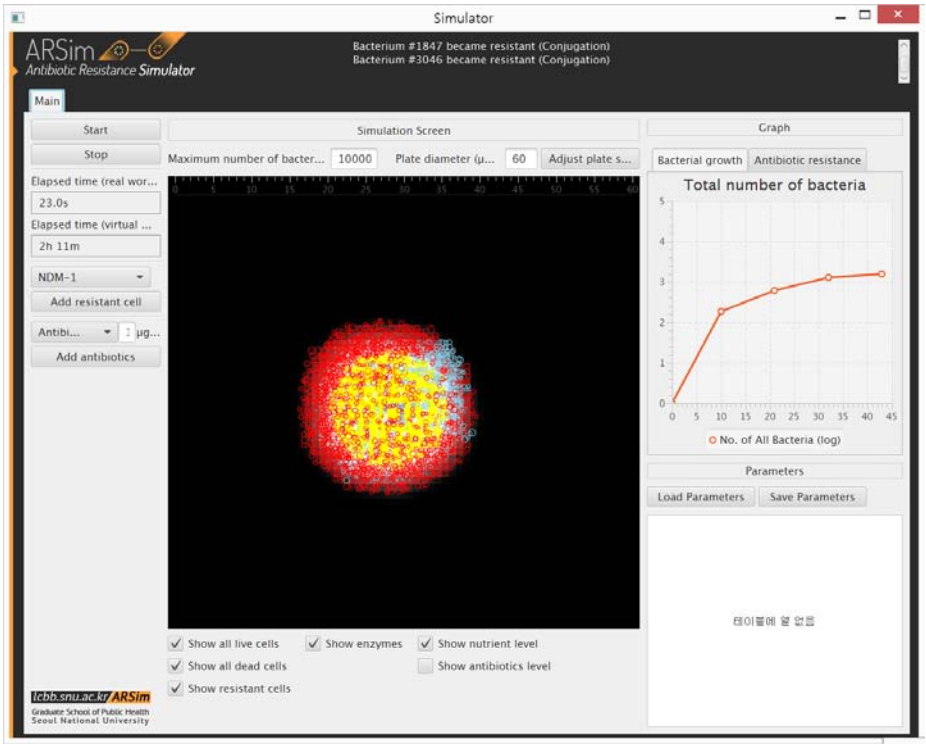


Figure 3.2. Screenshot of the simulation program ARSim during a bacterial growth simulation.

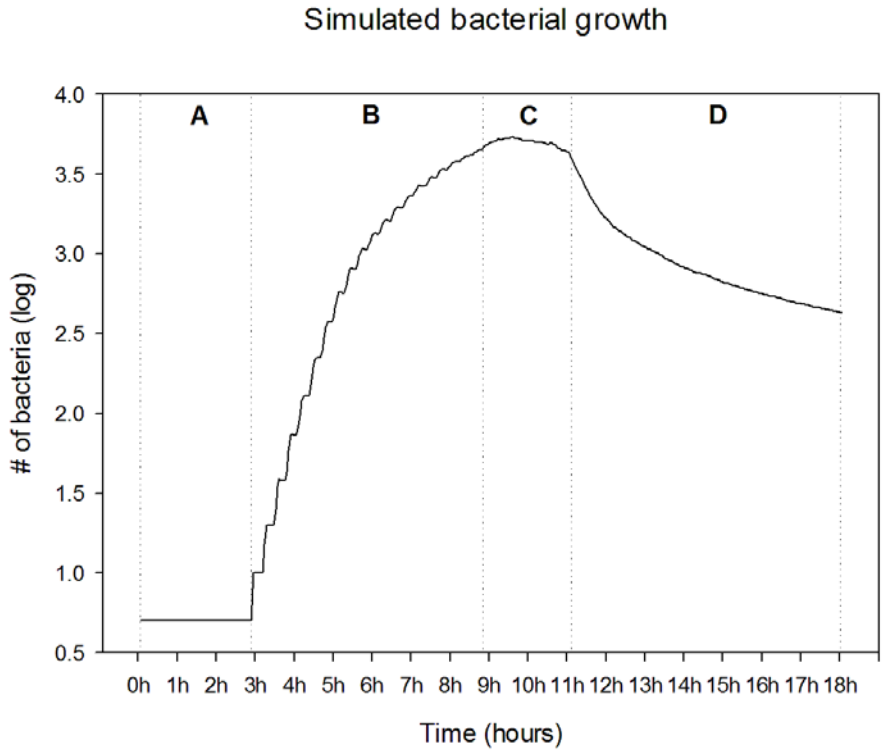


Figure 3.3. Simulation of the bacterial growth model. A simulation model of bacterial population growth. The results conform with the typical four phases of the bacterial growth curve which is depicted as sections A-B-C-D. Parameters are set as follows: Minimum cell diameter = 0.3 μm, Maximum cell diameter = 1.0 μm, Plate diameter = 60 μm, Nutrient level = 300, Diffusion coefficient = 0.5, Lag phase = 60, Amount of released nutrients from dead cells = 10, Generation time = 40 minutes

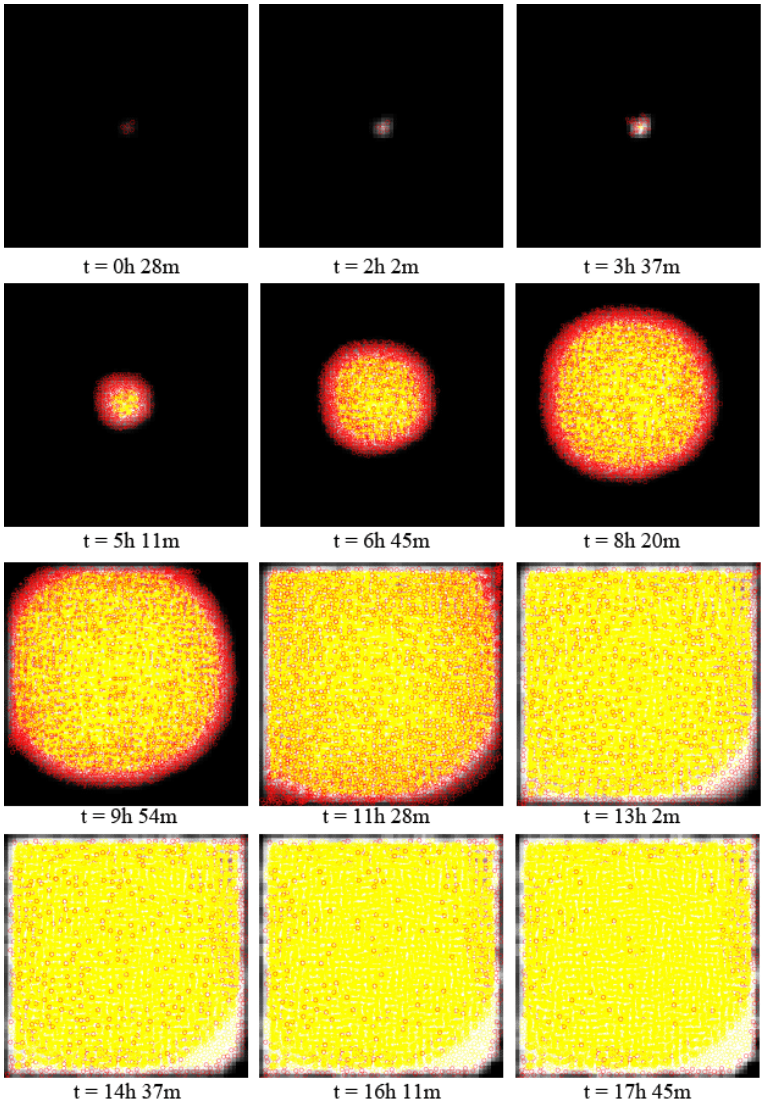


Figure 3.4. Visualization of the bacterial growth model. Visualization of the simulation of bacterial population growth (Figure 3.3). Simulation was performed for 3 minutes in real world and 18 hours virtually. Snapshots show the process of growth, division and colonization of bacterial cells within a virtual plate implemented in the simulation program.

Table 3.1 Parameters for the simulation of bacterial growth

Parameter group	Name	Value	Unit
Environment	Virtual plate patch size	0.1	μm
	Virtual plate full size	60.0	μm
	Diffusion coefficient	0.5	-
	Lag phase	60	-
	Initial nutrient level per patch	300	-
	Emitted nutrient level from dead cells	10	-
Bacteria	Initial number of bacteria	5	cells
	Minimum diameter	0.3	μm
	Maximum diameter	1.0	μm
	Generation time	40	minutes

3.2 Simulation of the antibiotic resistance model

For the second part of the simulation, a set of experiments associating the interactions of bacteria and antibiotics were conducted. We ran simulations based on the designed antibiotic resistance models on top of the bacterial growth model mentioned in the previous chapter. Simulations were conducted for two groups of bacteria where one group is fully susceptible to antibiotics and the other group is mixed with both antibiotic resistant and non-resistant bacteria.

3.2.1 Simulation without antibiotic resistant bacteria

We added antibiotics in growing bacteria without any type of antibiotic resistance and predicted the consequences of the simulation. Thus, parameters set for the bacterial and environmental factors were same as the previous experiment (Table 3.2). The antibiotic used in this simulation was a Carbapenem class Imipenem and the concentrations for this antibiotic were 0.05μg/ml and 0.1μg/ml each. These numbers were based on experiments of *K.pneumoniae* without resistance to carbapenems that had the MIC value of 0.094μg/ml against Imipenems (Yong et al., 2009). We assumed that with a concentration of 0.05μg/ml, bacteria will not react to antibiotics and in 0.1μg/ml which is higher than 0.094μg/ml, most bacteria will be killed by antibiotics.

We conducted two simulations for this experiment. The first was growing bacteria without adding antibiotics for 12 hours. For the second simulation, we added antibiotics after running the same simulation. As a result for the first simulation, the result was shown as a graph similar to the result of 3.1.1. For the second simulation, we added antibiotics of two different concentrations after approximately 7.5 hours of simulation. Antibiotics were

added to the same group of bacteria at the same given time with different concentrations of 0.05μg/ml and 0.10μg/ml each. Bacteria mostly survived when the concentration was 0.05μg/ml whereas bacteria were predicted to be annihilated in nearly 20 minutes after the simulation, when the concentration was up to 0.1μg/ml (Figure 3.5). Figure 3.6 visualizes the difference between concentrations where (A) shows the expansion of bacterial population when 0.05μg/ml is added and (B) shows instant death when t=7h48min when 0.10μg/ml of antibiotics is added.

3.2.2 Simulation with antibiotic resistant bacteria

The next step in our study was the simulation of antibiotic resistance in bacterial population that contains antibiotic resistant bacteria. We conducted simulations to predict the consequences of adding two kinds of different antibiotics when antibiotic resistant bacteria were present.

The first experiment was adding antibiotics at a given time into a population of carbapanem resistant *K.pneumoniae* bacteria with a NDM-1 gene and bacteria without any resistance. Imipenem was chosen for the carbapenem class antibiotic and the concentration was set to 16μg/ml. The concentration was set based on experiment results considering that MIC values for non-resistant *K.pneumoniae* and carbapenem resistant *K.pneumoniae* were 0.094μg/ml and 32μg/ml each, it was appropriate to set the concentration to a value between these numbers. Our assumption for this experiment was when antibiotics are added, non-resistant bacteria will nearly extinct and resistant bacteria on the other hand will start to increase dramatically.

Antibiotics were added after four hours of simulation. As a result, non-resistant bacteria became extinct in approximately 10 minutes, lowering the level of competition for nutrients and space among bacteria, which made bacteria with resistance grow and spread dramatically as expected (Figure 3.7,

Figure 3.8).

To provide a visualized output of the simulation process, a timer was set in the program to periodically capture screenshots of the simulation. Bacteria with no antibiotic resistance were colored in red, bacteria with resistance were colored in skyblue and dead bacteria were colored yellow. Since this experiment was focused on observing the changes in bacterial population and their resistance over time, adding antibiotics which should be appeared in the screenshot as a green background proportional to the concentration was set as transparent.

The second experiment was to run a simulation to observe the action mechanism of antibiotic resistance (Figure 3.9). The mechanism starts with acquiring antibiotic resistance through genetic mutation. As bacteria grow, horizontal gene transfer of these resistance genes to adjacent bacteria occurs through conjugation. Further, as bacteria divides, the resistance genes are transferred vertically to their daughter cells. Finally, when antibiotics are added to bacterial population, resistance mechanisms are set to produce enzymes called carbapenemase that is in charge of hydrolyzing the antibiotic molecules.

We were able to observe the horizontal gene transfer of resistance over time when antibiotic resistance genes were added during the bacterial growth. The vertical passing of resistance genes to daughter cells were also observed in cases of binary fission. After a certain amount of time when antibiotics were added, if the concentration of antibiotics was higher than a certain threshold, enzymes that decompose antibiotic molecules were produced. As seen in Figure 3.8, antibiotic resistant bacteria were colored in skyblue and the enzymes were depicted as blue colored dots. In this experiment the concentration of antibiotics was a critical factor therefore we painted antibiotics in green proportional to the concentration.

For the third part of the simulation, we added antibiotics to a bacterial

population mixed with both antibiotic resistant bacteria and non-resistant bacteria to predict the changes in population and determine the effectiveness of antibiotics to certain bacteria. As we have already done in previous experiments, carbapenem class antibiotics were used against CRE bacteria. In order to observe the difference of antibiotics used according to their parameters, Imipenem and Meropenem were used in each tests. The characteristics of antibiotics are represented in four parameters, which are the Michaelis constant (K_m) and the catalytic conversion efficiency or turnover rate (k_{cat}) from the Michaelis-Menten kinetics, the molecular mass and half - ife. The Michaelis constant and Turnover rate were set to 127μM, $10.8s^{-1}$ and 68μM, $4.0s^{-1}$ for Imipenem and Meropenem respectively. The molecular mass for each antibiotics were set to 299 da(g/mol) and 383 da(g/mol) and was used for calculating the concentration in the Michaelis-Menten kinetics equation. Also, MIC values of antibiotics against particular bacteria with or without resistance were collected from literatures and stored in the database so that the simulation tool can query the MIC value during simulations in situations when antibiotics and bacteria react based on user input bacteria and resistance. Parameters set for these simulations are listed in Table 3.3.

The third experiment was conducted with seven simulations. One simulation was an untreated control which is the same as the bacterial growth simulation without adding any antibiotics at all. Next simulations were consisted of three simulations with Imipenem and the other tree with Meropenem, which are both carbapenem class antibiotics. Simulations were each ran with three different concentrations of 16 μg/ml, 32 μg/ml, 64μg/ml. Concentration chosen for simulations were based on lab results of *K.pneumoniae* against Imipenem and Meropenem. The untreated control was used for the comparsion of the other six simulations. The results using Imipenem and Meropenem are shown in Table 3.4.

Simulations that used Imipenems showed instant decrease in the

population in three different concentrations of antibiotics(Figure 3.10, 3.11, 3.12, 3.13, 3.14). The degree of decrease was the least in 16 μg/ml and the most in 64 μg/ml. However, when 16 μg/ml of Imipnem was added, there was a rebound in growth after approximately 2 hours. This also occurred when the MIC value was 32 μg/ml, which is reported as the MIC of a NDM-1 producing *K.pneumoniae* against Imipenems, the time to rebound was predicted to be longer than the simulation with 16 μg/ml. When 64 μg/ml of Imipenem was added which is twice as high as the MIC for *K.pneumoniae*, the bacterial population was inhibited from growing as it has been almost eliminated. After six hours of simulation, the population of untreated control started to decrease (Figure 3.10). However, this was not seemed to be in any accordance with antibiotics, instead it was related to the depletion of available nutrients. This goes the same for the next hour and until the end of all three simulations including the untreated control, which was also found irrelevant to the aim of our experiment. We can claim that the first six hours of simulations from t=0 to t=6 are the results that we tried to focus on, and the graph of results which is in the form of time-kill curves were similar to a time-kill curve in a previous study (Worthington et al., 2012; Tang et al., 2014).

Additional simulations were conducted using other antibiotics. When Meropenem was added to the same bacterial population with both carbapenem resistant bacteria and non-resistant bacteria, the population decreased dramatically on all three concentrations 16 μg/ml, 32 μg/ml and 64 μg/ml. Very similar to the previous simulation with Imipenem, the overall population started to steadily increase after approximately two hours of simulation in concentrations of 16 μg/ml and 32 μg/ml, and annihilated when the concentration was 64 μg/ml(Figure 3.15, 3.16, 3.17, 3.18). The predicted time-kill curve was overall similar to the graph using Imipenem but the rate of increase appeared to be slower when the concentration was 32 μg/ml. Our

estimation for this difference is related to the parameters that explain the catalytic efficiency, k_{cat}/K_m. Since parameters for k_{cat} and K_m of Imipenems were 10.8 and 127, $(k_{cat}/K_m)_{Imipenem}$ = 10.8/127 = 0.085, and k_{cat} and K_m for Meropenems were 4.0 and 68 which would be also put as $(k_{cat}/K_m)_{Meropenem}$ = 4.0/68 = 0.059, we can assume that Meropenem has a lower efficiency than Imipnem against *K.pneumoniae* because the k_{cat}/k_M has a smaller value. This results in a lowered speed of degrading antibiotics by carbapenemase enzymes which eventually causes the delay of bacterial growth.

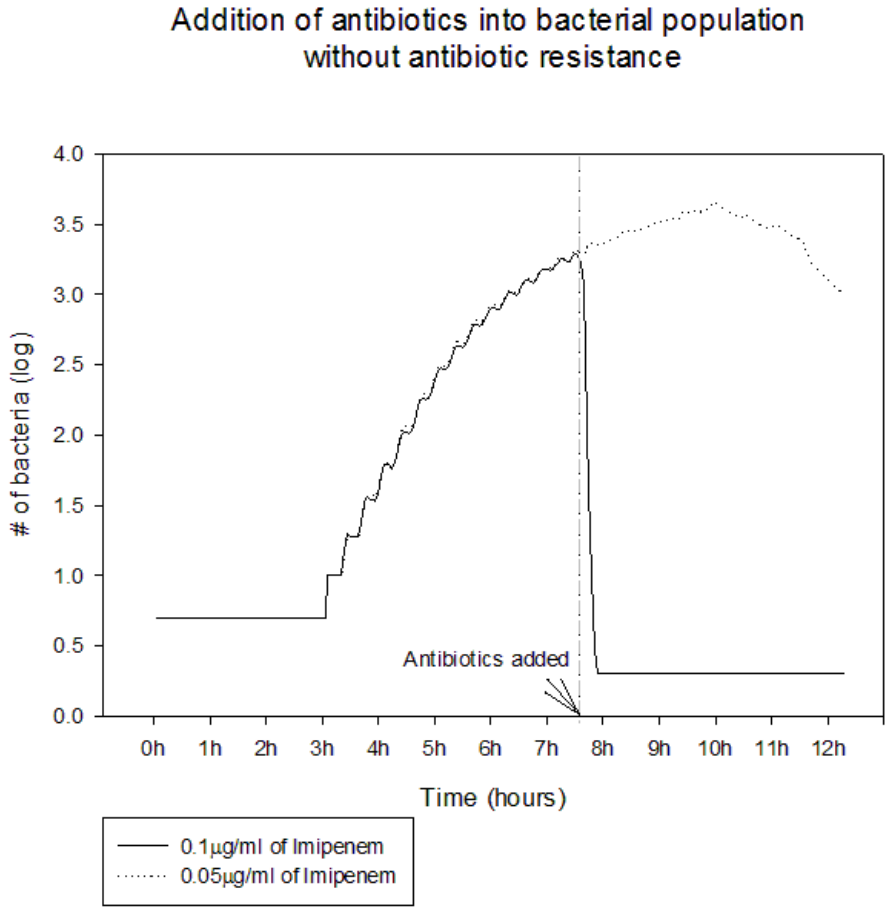


Figure 3.5. Simulation of the antibiotic-bacteria interaction model. A simulation model of the interactions between antibiotic molecules and bacteria without antibiotic resistance. Simulations were performed twice, each with 0.05µg/ml and 0.1µg/ml of Imipenem, a carbapenem class antibiotic, added to the bacterial population at a same given time.

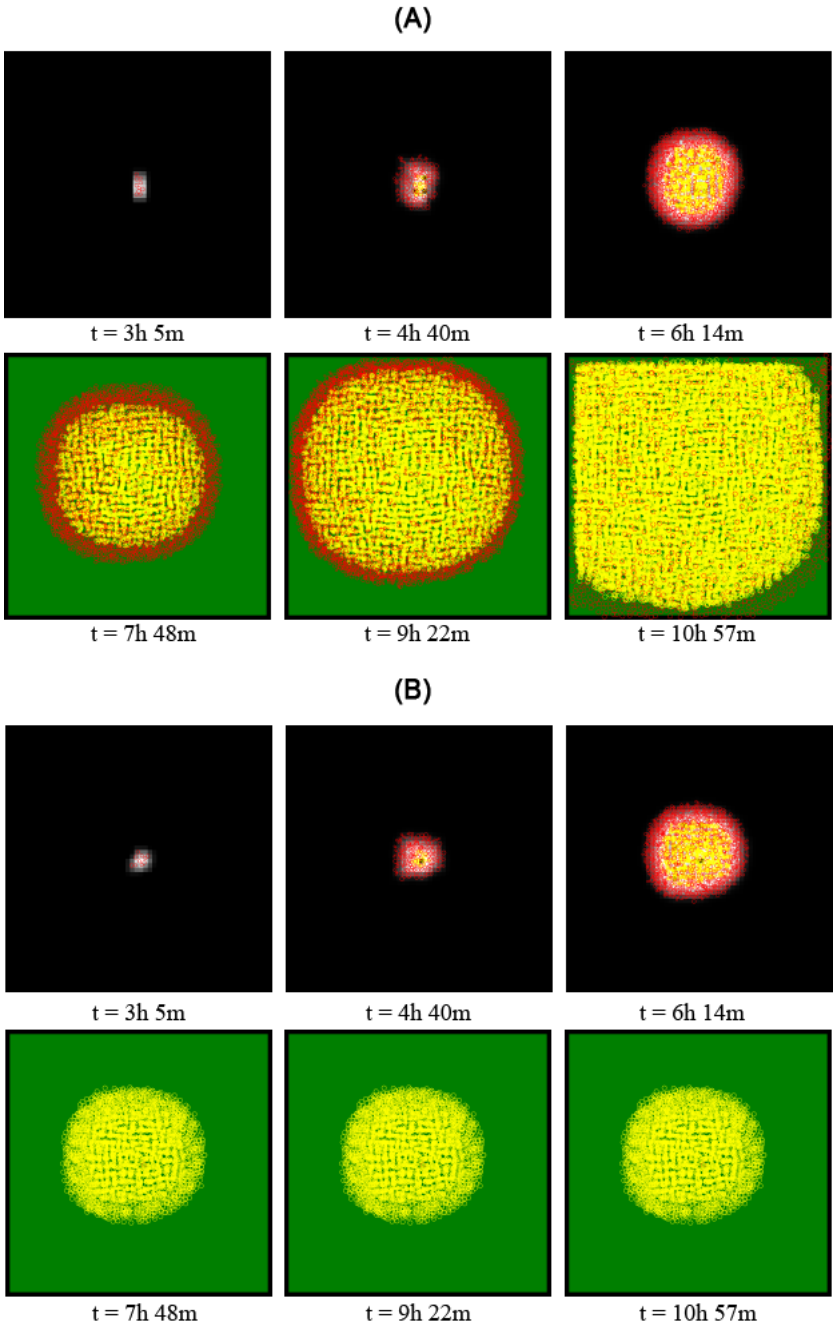


Figure 3.6. Visualization of antibiotic-bacteria interaction model. As shown in Figure 3.5, two simulations were performed with the same antibiotic with different

concentrations. (A): 0.05 μg/ml , (B): 0.1 μg/ml . In both (A) and (B), antibiotics were added after approximately 7 hours of simulation which is depicted in the snapshots as the green colored background. (A) shows continuing bacterial growth due to insufficient amount of antibiotics whereas (B) shows instant death of bacterial cells minutes after antibiotics being added.

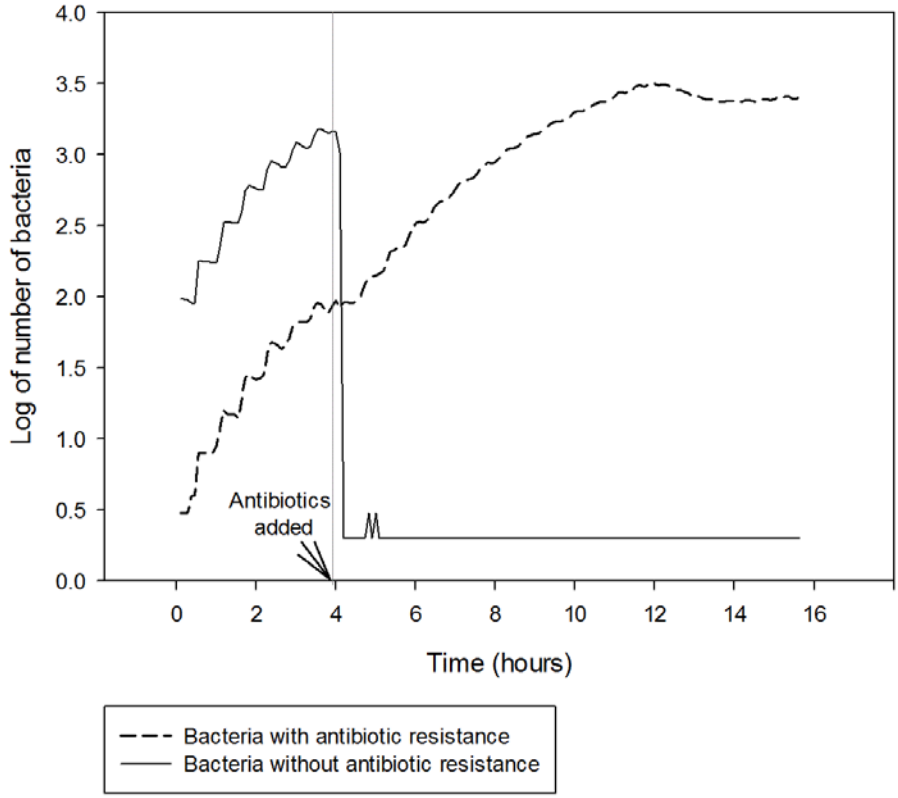
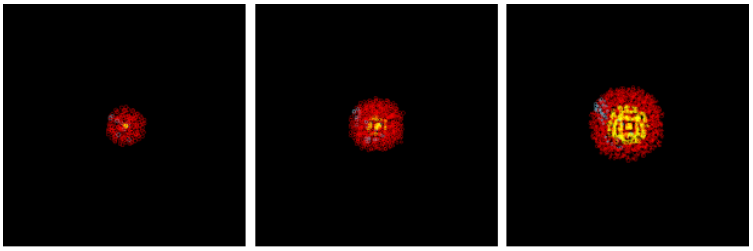
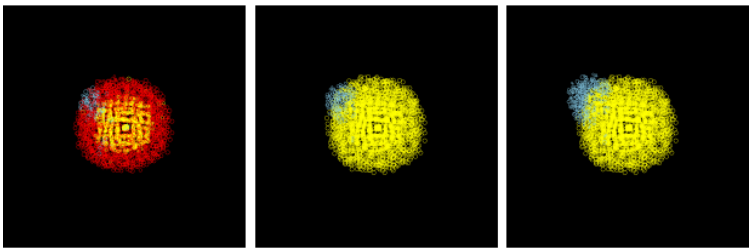


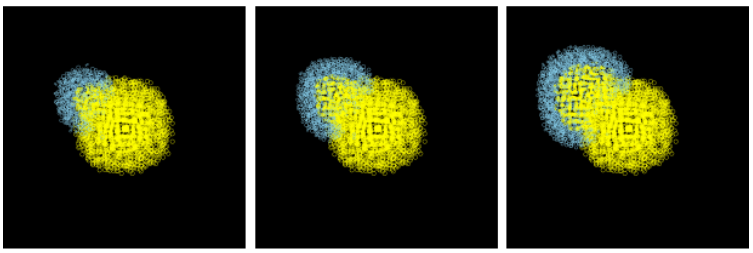
Figure 3.7. Simulation of antibiotic addition performed on bacterial population with and without antibiotic resistance. Result of the simulation was observed in two steps. First, the total number of bacterial cells with antibiotic resistance was counted to observe the correct implementation of resistance mechanisms. Second, the total number of bacterial cells without antibiotic resistance was counted in order to observe the consequences of the antibiotic addition, which resulted to be contrary to the first experiment. Most bacteria survived and continued to grow when 16µg/ml of Imipenem was added to bacterial cells with NDM-1 carbapenem resistance gene. Population without resistance genes were bactericided by the antibiotic.



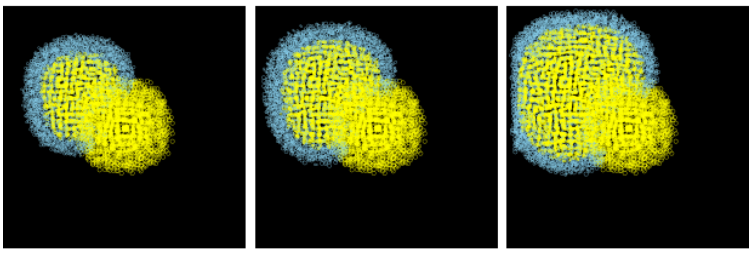
t = 1h 0m t = 2h 0m t = 3h 0m



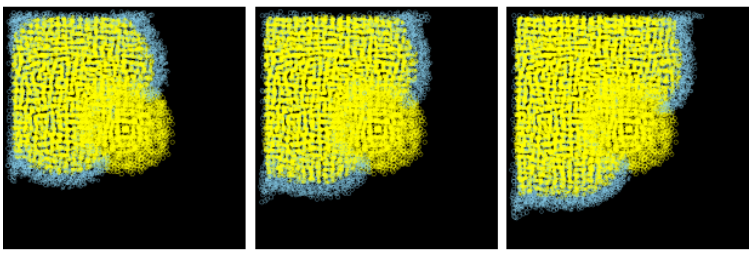
t = 4h 0m t = 5h 0m t = 6h 0m



t = 7h 0m t = 8h 0m t = 9h 0m



t = 10h 0m t = 11h 0m t = 12h 0m



t = 13h 0m t = 14h 0m t = 15h 0m

Figure 3.8. Visualization of antibiotic resistance gene transfers in bacterial population. As antibiotics are added after 4 hours of simulation(t=4h0m), the number of antibiotic resistant bacteria steadily increases while non-resistant bacteria fail to survive. Antibiotic resistant bacteria are represented in skyblue, non-resistant bacteria in red, dead bacteria in yellow.

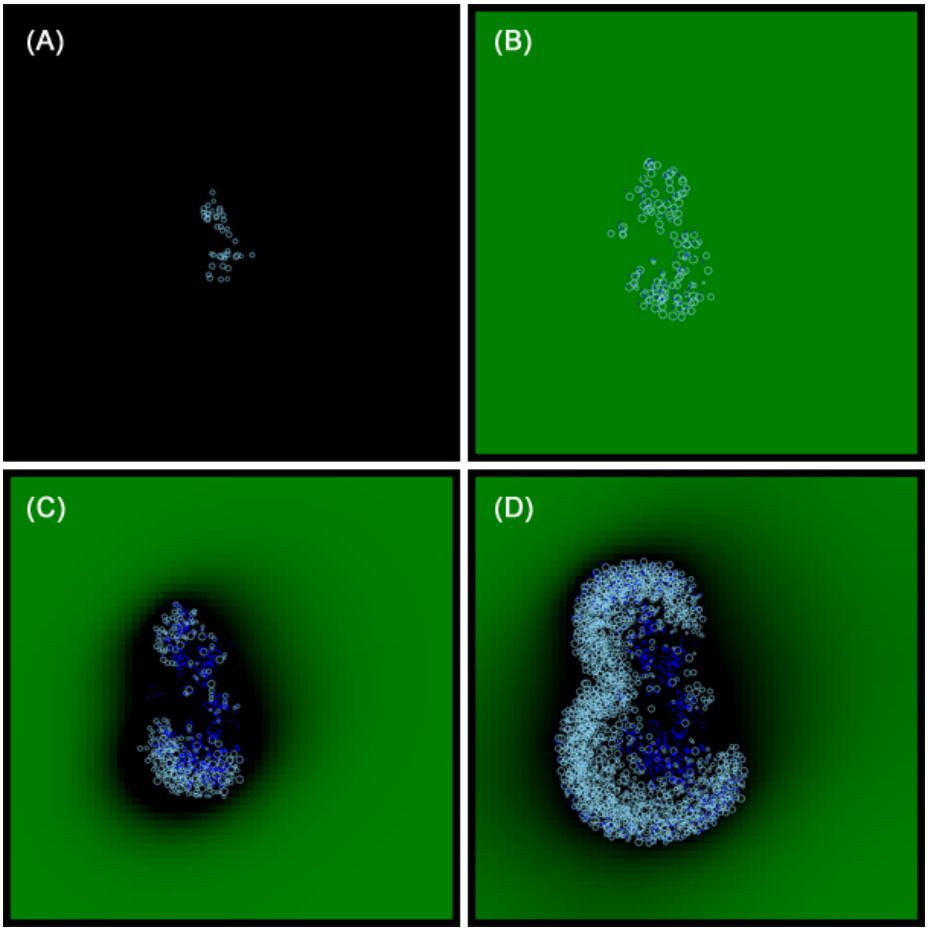


Figure 3.9. Visualization of antibiotic resistance mechanisms. Bacteria with antibiotic resistance are represented in skyblue, green represents concentration of antibiotics and blue represents antibiotic hydrolyzing enzymes produced from bacteria. (A) Antibiotic resistance genes are spread throughout the population by mutation and conjugation, (B) Addition of antibiotics triggers the resistance gene to produce enzymes, (C) Enzymes react with antibiotics and (D) Antibiotic resistant bacteria increases dramatically and forms a colony. For better understanding and clear visualization of these mechanisms, bacteria without antibiotic resistance along with dead cells were not displayed.

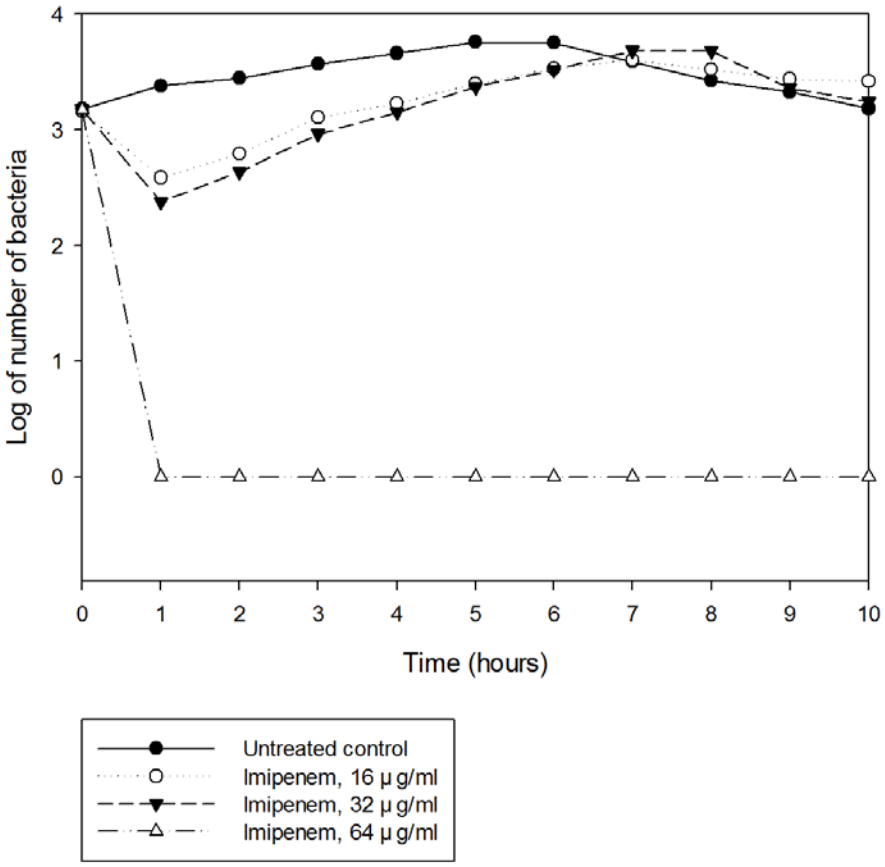


Figure 3.10. Predicted time-kill curves for Imipenem against carbapenem-resistant *K. pneumoniae* with resistance gene NDM-1. Parameters include K_m = 127µM, k_{cat} = 10.8s^{-1}, Molecular mass = 30000da, Half life = 206min, MIC = 32µg/ml.

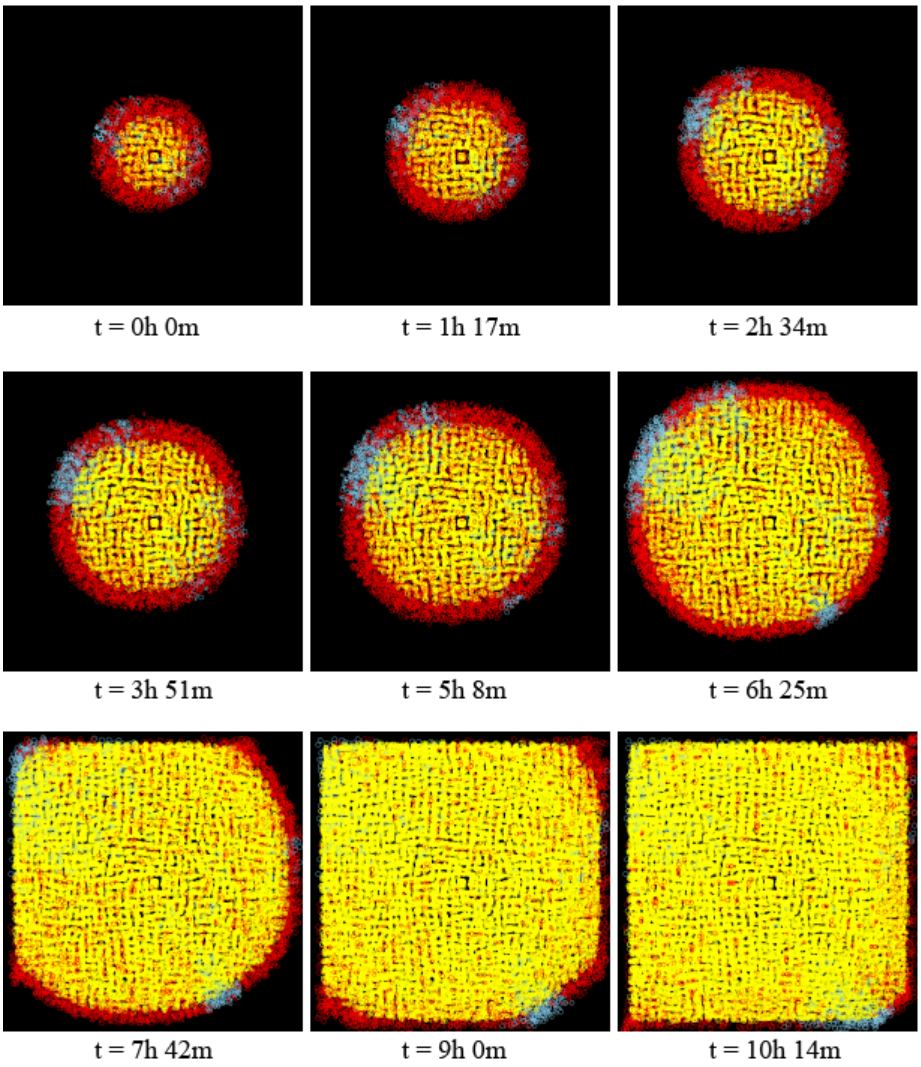


Figure 3.11. Visualization for no antibiotic treatment against *K. pneumoniae* with resistance gene NDM-1.

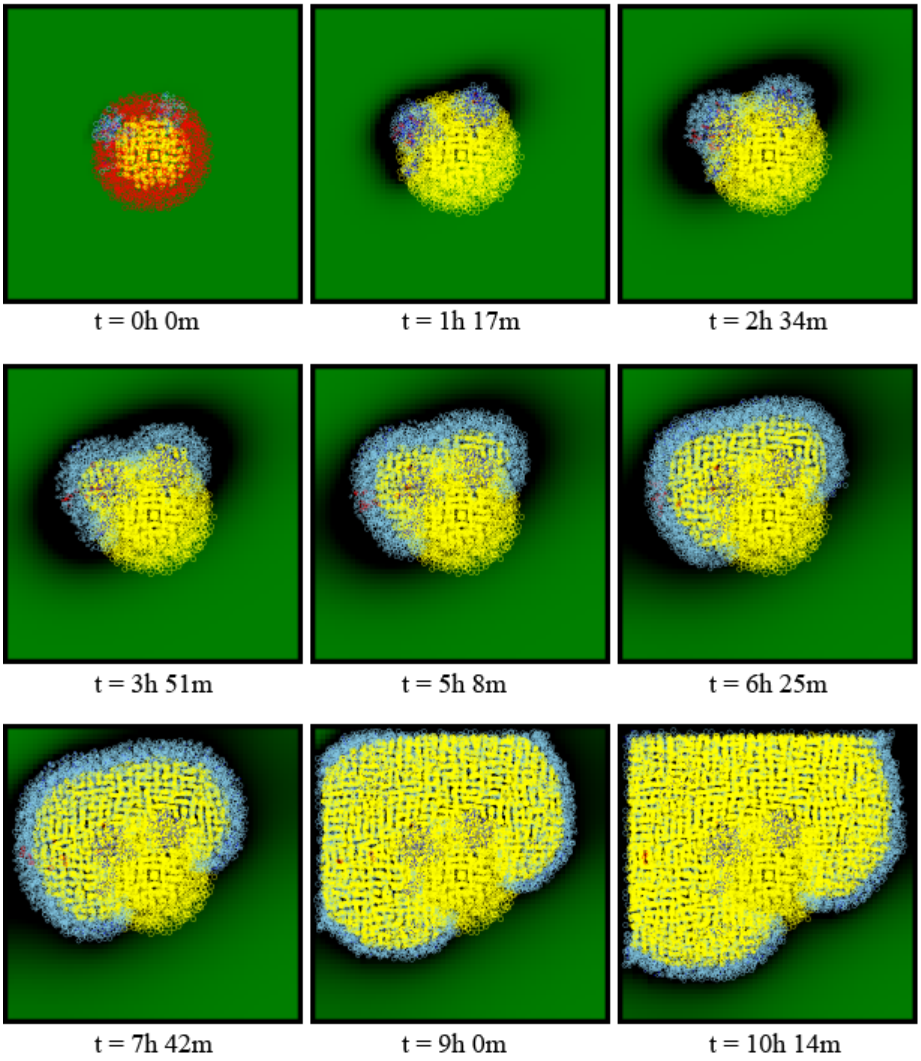


Figure 3.12. Visualization for 16µg/ml of Imipenem against carbapenem-resistant *K. pneumoniae* with resistance gene NDM-1.

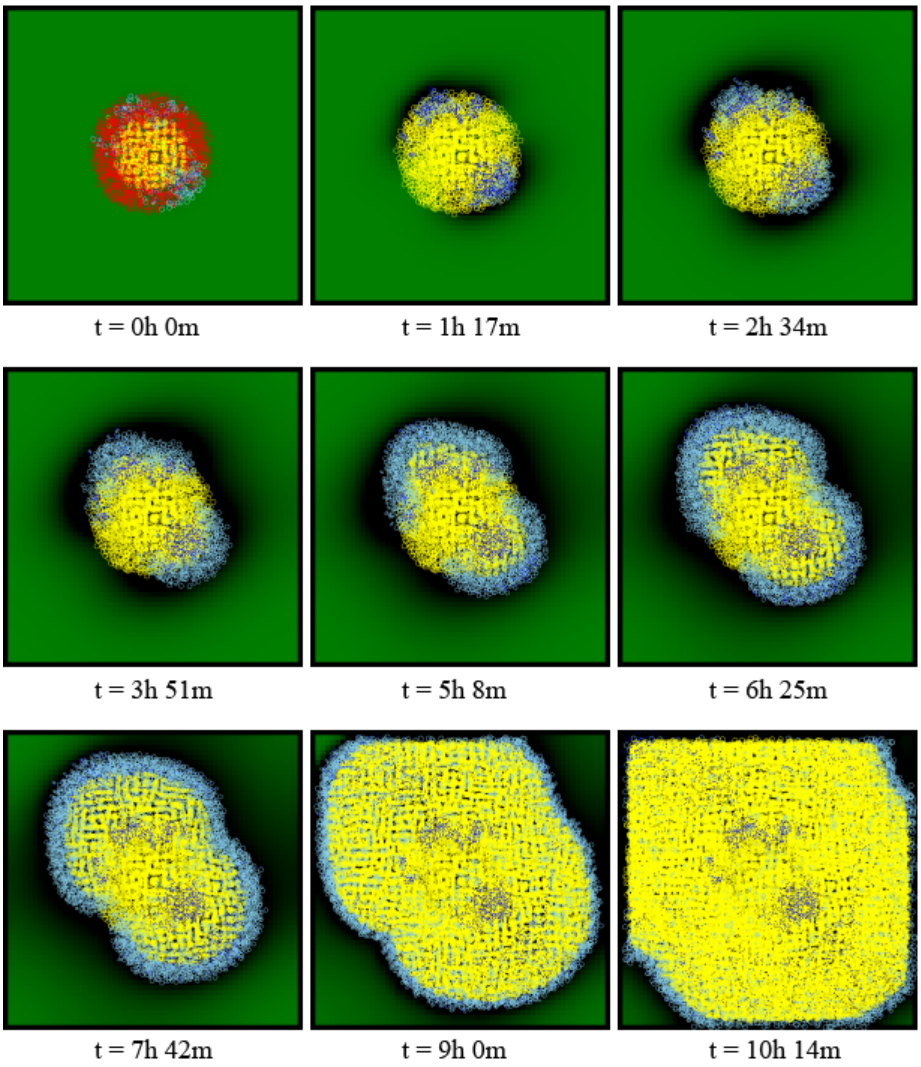


Figure 3.13. Visualization for 32µg/ml of Imipenem against carbapenem-resistant *K. pneumoniae* with resistance gene NDM-1.

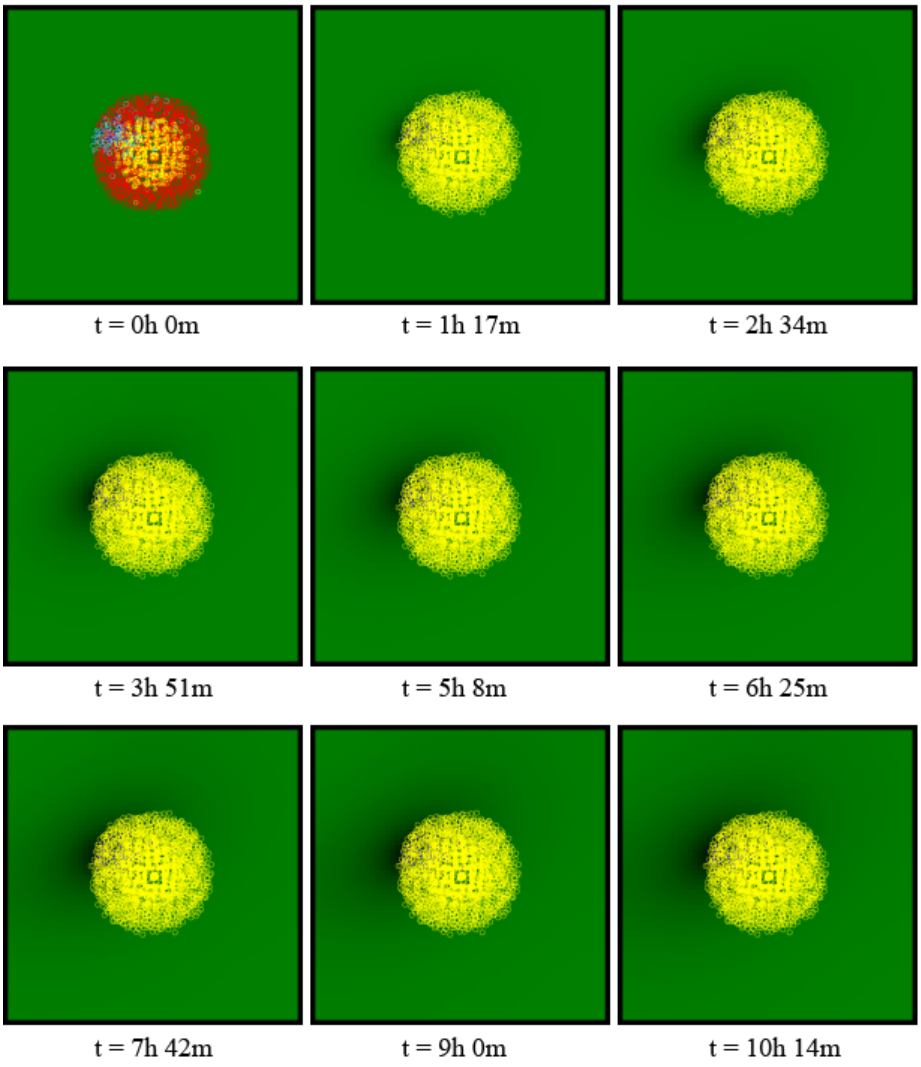


Figure 3.14 Visualization for 64µg/ml of Imipenem against carbapenem-resistant *K. pneumoniae* with resistance gene NDM-1.

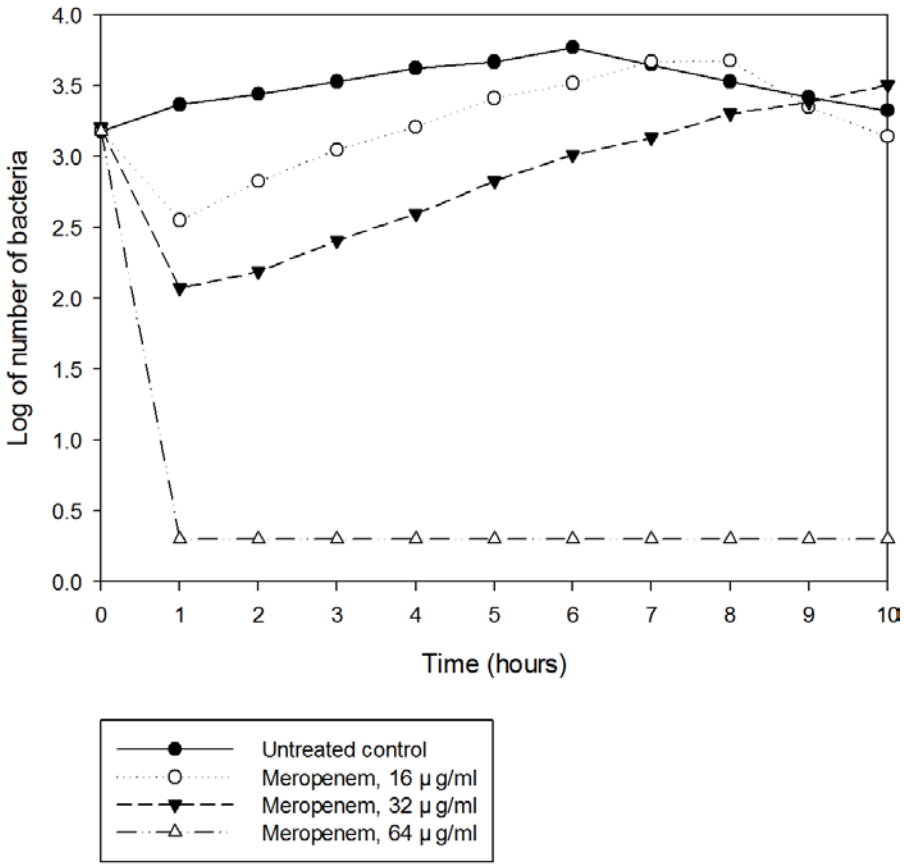


Figure 3.15. Predicted time-kill curves for Meropenem against carbapenem-resistant *K. pneumoniae* with resistance gene NDM-1. Parameters include K_m = 68µM, k_{cat} = 4.0s^{-1}, Molecular mass = 30000da, Half life = 206min, MIC = 32µg/ml.

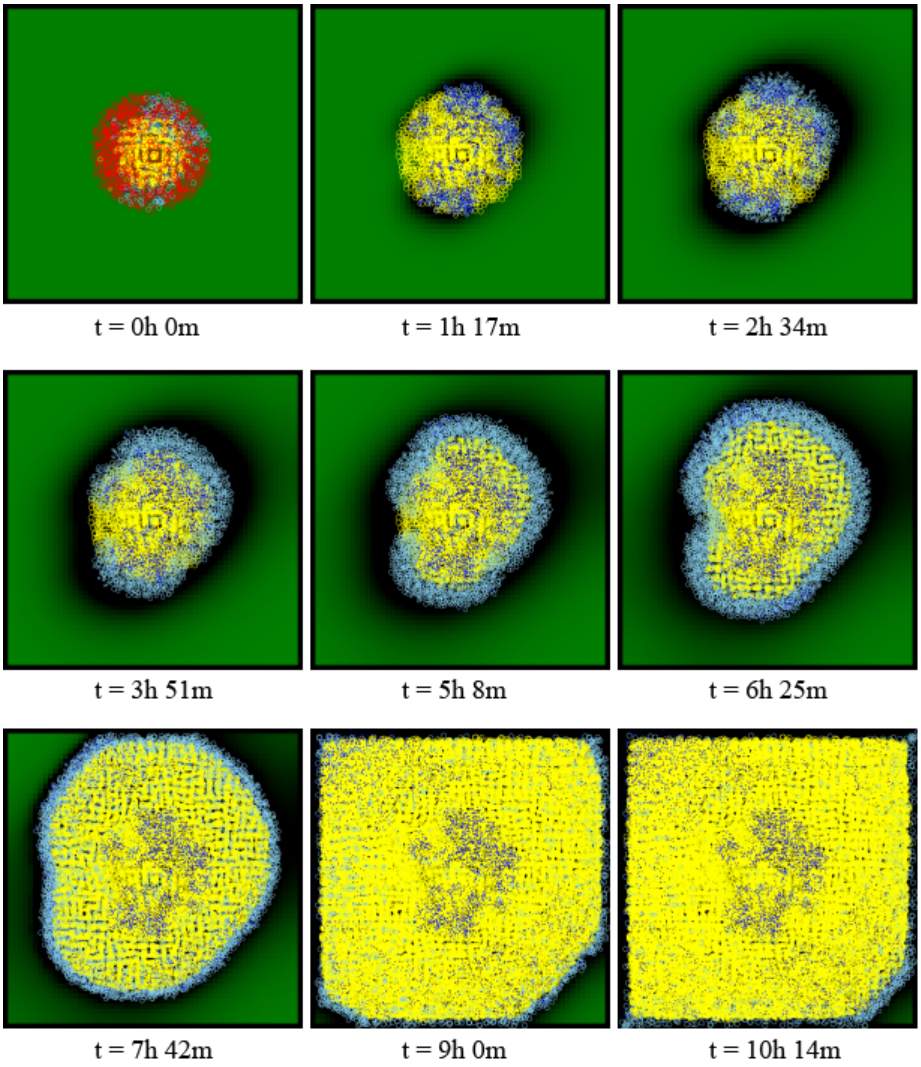


Figure 3.16. Visualization for 16μg/ml of Meropenem against carbapenem-resistant *K. pneumoniae* with resistance gene NDM-1.

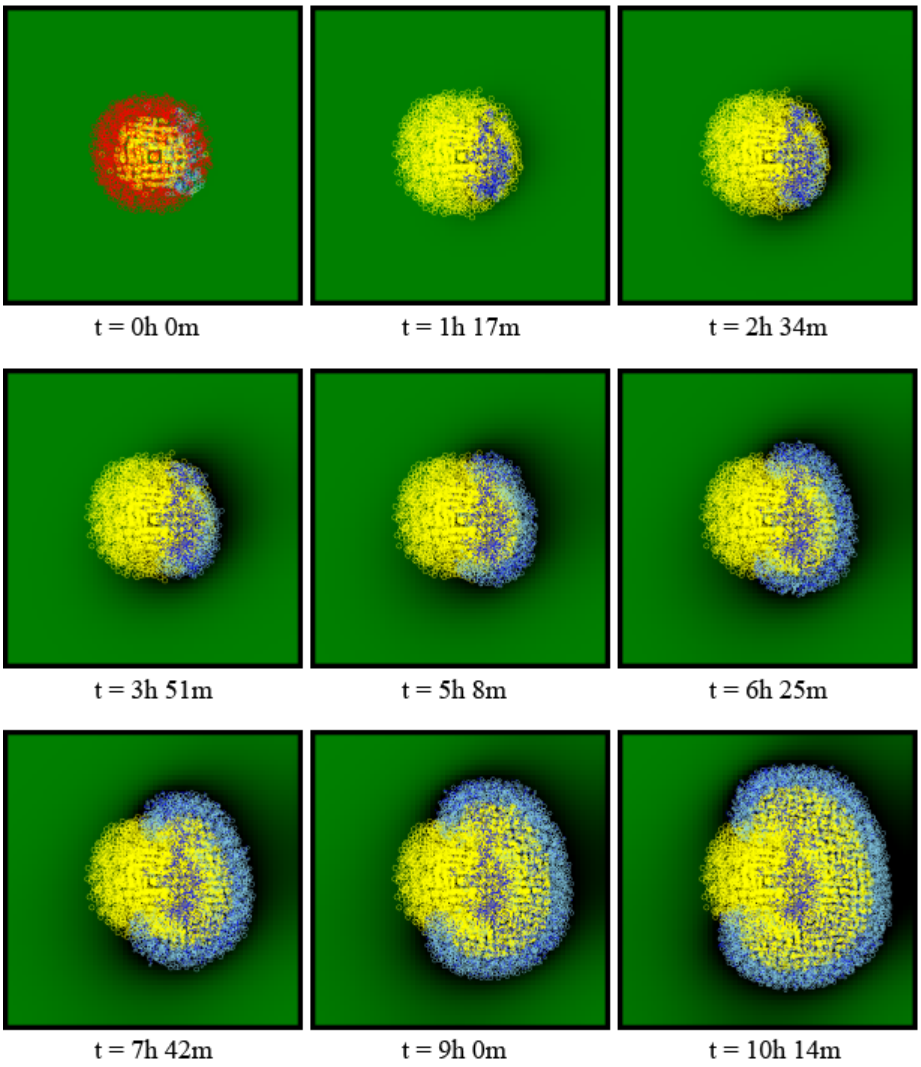


Figure 3.17. Visualization for 32µg/ml of Meropenem against carbapenem-resistant *K. pneumoniae* with resistance gene NDM-1.

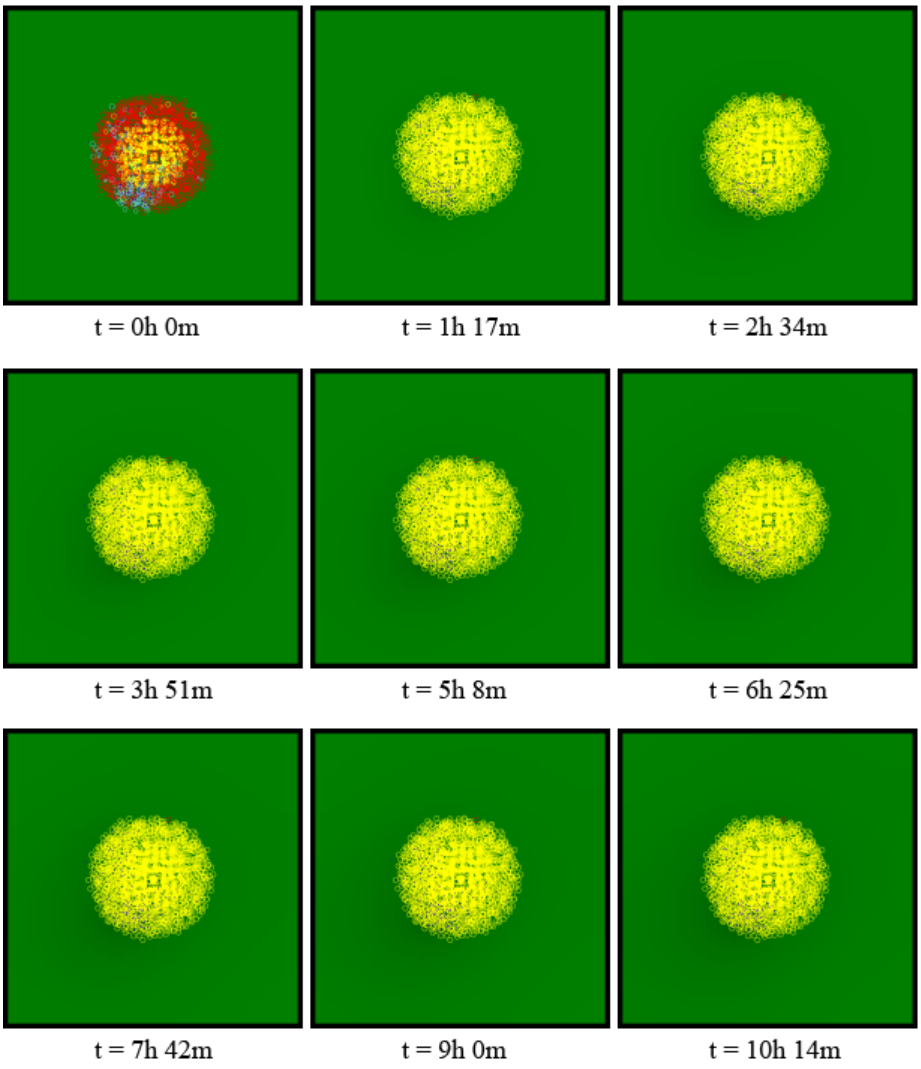


Figure 3.18. Visualization for 64µg/ml of Meropenem against carbapenem-resistant *K. pneumoniae* with resistance gene NDM-1.

Table 3.2 Parameters for the simulation of antibiotic resistance without resistance genes

Parameter group	Name	Value	Unit
Environment	Virtual plate patch size	0.1	μm
	Virtual plate full size	60.0	μm
	Diffusion coefficient	0.5	-
	Lag phase	60	-
	Initial nutrient level per patch	300	-
	Emitted nutrient level from dead cells	10	-
Bacteria	Initial number of bacteria	5	cells
	Minimum diameter	0.3	μm
	Maximum diameter	1.0	μm
	Generation time	40	minutes
Antibiotics	Name of antibiotic	Imipenem	-
	Molecular mass	299	g/mol
	MIC against bacteria	0.094	μg/ml

Table 3.3 Parameters for the simulation of antibiotic resistance with resistance genes

Parameter group	Name	Value	Unit
Environment	Virtual plate patch size	0.1	μm
	Virtual plate full size	60.0	μm
	Diffusion coefficient	0.5	-
	Lag phase	60	-
	Initial nutrient level per patch	300	-
	Emitted nutrient level from dead cells	10	-
Bacteria	Initial number of bacteria	1000 >	cells
	Minimum diameter	0.3	μm
	Maximum diameter	1.0	μm
	Generation time	40	minutes
Enzymes	Enzyme encoding gene	NDM-1	-
	Molecular mass	30000	g/mol
	Half life	206	minutes
Antibiotics	Name of antibiotic	Imipenem	-
	Molecular mass	299	g/mol
	Michaelis constant(K_m)	127	μM
	Turnover rate(k_{cat})	10.8	s^{-1}
	MIC against bacteria	32	μg/ml
	Name of antibiotic	Meropenem	-
	Molecular mass	383	g/mol
	Michaelis constant(K_m)	68	μM
	Turnover rate(k_{cat})	4.0	s^{-1}
	MIC against bacteria	32	μg/ml

Table 3.4 Total number of *K. pneumoniae* bacteria after addition of carbapenem antibiotics

Antibiotic	Conc.	Log of number of bacteria at:				Change in number of bacteria after:		
		0h	1h	5h	10h	Δ1h	Δ5	Δ10h
Untreated control		3.18	3.37	3.67	3.33	+0.19	+0.49	+0.15
Imipenem	16	3.16	2.59	3.40	3.42	-0.58	+0.23	+0.26
	32	3.17	2.38	3.37	3.25	-0.80	+0.20	+0.07
	64	3.17	0	0	0	-3.17	-3.17	-3.17
Meropenem	16	3.18	2.55	3.41	3.14	-0.63	+0.23	-0.04
	32	3.21	2.08	2.83	3.51	-1.13	-0.37	+0.30
	64	3.18	0.30	0.30	0.30	-2.88	-2.88	-2.88

CHAPTER IV.

DISCUSSION AND CONCLUSION

4.1 Discussion

In this study, we conducted virtual experiments with simulation models of bacterial growth and antibiotic resistance. Our study has the following three noticeable characteristics compared to previous studies.

First, we developed the whole set of simulation models and tools from scratch. The models were consisted of molecular objects from bacterial cells, enzymes to antibiotics which all have been modeled and programmed into the ARSim simulation tool. Furthermore, it was hard to find in previous studies that provide graphical interfaces and visual outputs for results.

Second, we implemented both horizontal and vertical gene transferring mechanisms of genes involved in antibiotic resistance. The horizontal gene transfer mechanism was implemented as passing genes from plasmids in bacterial cells to other cells when they are close enough to be connected by pilis. The vertical gene transfer was implemented by passing resistance genes to inheritors when cells divide.

Lastly, the simulation tool was developed for researchers in laboratory or clinical settings to be able to use with ease. It was developed in Java to be run on multiple platforms and the simulation parameters can be adjusted in minimum hardware requirements. Although it is widely considered that simulations are typically run on workstations or servers with parallel processing environments, it is worth noticing that this program was written on top of a relatively slow JVM(Java Virtual Machine) framework and does its work by optimizing data structures and algorithms. Using Java has an

advantage on its independence on operating systems, but runs on the Java Virtual Machine which is known to hinder the speed of compiled programs compared to other directly run programming languages such as C and C++. In order to resolve efficiency issues, we implemented Quadtrees for the data structure of bacteria and enzymes and minimized the transactions with the database. Also, parameters being applied to the simulation model were to be saved and loaded in JSON format for the ease of use.

Limitations of the study include the complexity of computational modeling, difficulty of implementing biological mechanisms, and the limited functionality and performance of the simulation tool.

In the perspective of biological mechanisms of bacteria and antibiotics, the main difficulty was deciding which parameters to use and exploring the scientific exactitude behind them. When designing the model of bacterial growth or its antibiotic resistance, it was practically impossible to implement all the physical actions and biochemical pathways underneath, so our choice was to use basic biochemical equations and parameters based on results of lab experiments. We made rules based on these data, however it was difficult to assess the accuracy and reality of the results. Therefore, we decided to make modules of the biological structure to objects based on their importance instead of implementing all aspects and ran simulations with input values that are same as values found in literatures. Also, various biological principles, such as the rate of production of enzymes when antibiotics are added to bacteria and how a dead bacterial cell affects the whole population were not found in previous literatures, and minimum inhibitory concentration values of antibiotics were sometimes different among studies which may lead to a defect in the outcome of simulation results.

Looking at the functional side of the simulation program, there were restrictions in the variety of parameters. We could not find evidence of the consequences when the temperature, acidity, kinds and amounts of nutrients

are changed during bacterial growth, so we had to exclude these parameters. All parameters regarding antibiotics were also simplified due to the same reason. Therefore, the unique advantage of simulation studies which is adjusting a variety of parameters and observing the outcome was restricted in some degree. However, the program itself was implemented with flexibility to use parameters, so when new antibiotics are to be added, implementing the mechanisms of actions based on literatures will make it possible to run simulations with the new drug. This flexibility lets this program to be open to further advancements.

4.2 Conclusion

The principal aims of this study were to design realistic models of bacterial growth and antibiotic resistance, implement the models to observe and predict the effects of antibiotics and compare them with previously conducted laboratory experimental results. We were able to design a bacterial population growth model and insure the validity by comparing the results with the four phases of bacterial growth. For the antibiotic resistance model, we implemented mechanisms of actions and interactions performed by the bacteria, enzymes and antibiotic molecules. Despite the lack of evidence, due to the difficulty of finding lab results conducted with the same bacterial strains and antibiotics as ours, we claim that the results of our simulation are fairly persuasive considering all underlying biological mechanisms.

There reside various public health issues currently in antibiotic resistance. First is the lack of preparation for newly evolved resistant bacteria. In contrast to antibiotic resistance, influenza viruses are strictly analyzed by the WHO(World Health Organization) and candidates of mutants that may cause global influenza pandemic are predicted periodically which leads to the development of vaccines to fight the predicted subtype. However, in a majority of cases in antibiotic resistance, there exist countless number of species that may evolve to become resistant to antibiotics in a variety of ways. Most of all, there cannot exist a single vaccine, such as an influenza vaccine, that work on all bacterial species. This may cause more problems since research, development and clinical trials of discovering new antibiotics involves heavy financial risk and the return on investment is not expected to be high considering the usage pattern of antibiotics where the drug should be used within a short time to prevent additional acquirement of resistance and continuous use of antibiotics is harmful. New methodologies are needed to be developed for finding new antibiotics and computational approaches used in

this study may provide a suitable alternative method during the research process of the drug development. For example, when a new strain of resistant bacteria is isolated, we can first analyze the sequence of the genome and copy the sequence into the simulation program. If the sequence contains genes that are identical or similar to known resistance genes, the program will start the simulation with the corresponding parameters stored inside the database. It will also be available to adjust parameters of already known resistant bacteria and observe the outcomes, compare it to lab experiments, which will provide a supplementation to existing experiment methods and perhaps replace the conventional procedures required for antibiotic resistance research in some cases. Predicted outcomes will be filtered out through constant comparison with lab results and stored into the database. Parameters derived from these procedures will be used in future simulations with a more robust evidence-based approach.

CHAPTER V. SUMMARY

The purpose of this study was to design, implement and run simulations based on models of bacterial growth and its resistance to antibiotics in the molecular level. The ultimate goal using the developed models and simulation tool was to find the proper antibiotics against bacteria by predicting the effects and consequences of adding antibiotics into a group of resistant and non-resistant bacteria.

We designed a computational model of carbapenem resistant *Klebsiella pneumoniae* strain with containing a NDM-1 gene based on individual based modeling methods then applied parameters to the model with values derived from previous lab experiments and literatures. Models of antibiotics, enzymes and the environment were also implemented. To run simulations based on these models, a simulation program named ARSim was developed. This program was built with high flexibility so that users can run simulations by setting their own parameters. Two main experiments were conducted using this program. The first experiment was the accurate implementation of bacterial growth, and the second was the observation and prediction of antibiotic resistance on top of the first experiment.

For the first experiment, we ran simulations of the bacterial growth model. The simulation was conducted for approximately 18 virtual hours and results showed that the simulated growth curve in some degree matched the theoretical four phases of bacterial growth. The lag phase was observed for the first 3 hours, exponential phase for the next 6 hours, stationary phase for 2 hours, and followed by was the death phase for 7 hours. For simulations on antibiotic resistance, we designed the resistance gene transfer models both horizontally through plasmids and vertically by inheriting. We chose

carbapenem class antibiotics Imipenem and Meropenem for the antibiotics model based on lab results from other studies. Carbapenemases, enzymes that are encoded from bacteria with NDM-1 genes, were chosen as the enzyme model that reacts with antibiotics. For the first experiment, we added antibiotics to a group of bacteria without antibiotic resistance. The results were as expected that the complete population of bacteria were instantly dead. Next, we added antibiotics to a bacterial population with mixed genes either resistant or not resistant to antibiotics. A distinguishable factor from the previous experiment was running the simulation with the change of antibiotic concentrations. Results were depicted as time-kill curves and turned out to be different for each level of concentration. We determined the validity of this simulation result through a comparison with previously conducted laboratory experimental results.

For further improvements of the simulation model, accurately validated data from traditional lab experiments are essential. Although simulation models are designed based on theoretical knowledge and principles, parameters that are actually used in the simulation must be able to properly reflect the phenomena that occur in the real world. As mentioned earlier, simulation methods provide more options to increase efficiency in biological experiments. However, these computational approaches do not exist to replace traditional lab experiments rather to complement each other. Considering that only one antibiotic was used during each simulation in this study, we can make additional improvements to the study by adding two or more antibiotics simultaneously to predict the combination effect of antibiotics (Tängdén et al., 2014). More efforts must be put on these kinds of experiments where researchers can maximize the utilization of simulation methods to overcome time or cost issues. Also, as gene or genome sequencing costs decline each year, we expect to see more sequencing cases of bacterial strains in the

clinical field. We should also prepare to use this sequence data in our program to provide the most similar model based on phylogenetic trees and eventually run simulations using the model. We hope the simulation models and tools implemented in this study provide better options for researchers with a new way of fighting antibiotic resistance.

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ABSTRACT (Korean)

박테리아의 항생제 내성에 대한 시뮬레이션 연구

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항생제 내성은 인체에 감염되어 질병을 일으키는 박테리아, 곰팡이, 바이러스 등의 다양한 병원성 미생물들이 이들을 치료하는 것으로 알려진 항생제에 내성을 갖게 되는 현상을 일컫는다. 항생제 내성은 국가의 지리적 여건 혹은 사회경제적 수준에 관계없이 발발하기 때문에 잠재적으로 더욱 심각한 보건학적 위협이며, 감염 경로가 다양하기 때문에 보건학적인 중재가 쉽지 않다. 전세계적으로 봤을 때 항생제 내성균에 의한 감염은 가파른 증가 추세이며, 항생제 내성 감염의 확산을 예방할 수 있는 연구와 정책이 필수적이다. 항생제 내성에 대응하기 위하여 전산적인 기법이 많이 활용되고 있는데, 특히 시간과 비용을 줄이고 다양한 연구를 효율적으로 시도해보기 위하여 컴퓨터 시뮬레이션을 유용하게 활용할 수 있다. 본 연구에서는 Individual based Modeling을 이용하여 박테리아의 생장과 항생제 내성에 대한 모델을 설계하였고, 이 모델에 대한 시뮬레이션을 수행하기 위하여 ARSim이라는 프로그램을 개발하였다. ARSim을 통해 박테리아를 생장시키는 실험과 항생제에 내성이 있는 집단, 그리고 내성이 없는

집단에 항생제를 더해보는 실험을 수행하였다. 박테리아의 생장에 대한 실험에서는 박테리아의 종을 *K.pneumoniae*으로 가정하였고, 기존 연구에서 얻어진 파라미터 값을 미리 설정하였다. 시간에 따른 박테리아 개체수의 변화를 예측한 결과, 생장유도기-대수증시기-정지기-사멸기로 이어지는 박테리아의 생장곡선 4단계를 따르는 형태로 나타났다. 두번째 실험에서는 항생제의 효과를 관찰하기 위하여 Carbapenem 계열 항생제인 Imipenem을 항생제 내성이 없는 일반 박테리아 집단에 더하였다. 그 결과, Imipenem 0.05μg/ml 이 더해진 집단은 생장을 계속하였고, 0.10μg/ml 이 더해진 집단은 생장을 멈추고 사멸하였다. 또한 항생제에 내성이 있는 집단과 없는 집단에 각각 16μg/ml, 32μg/ml, 64μg/ml의 Imipenem과 Meropenem을 더해본 결과, 각 농도별로 생장이 지연되는 현상과 일정 농도 이상일 때 박테리아가 모두 사멸되는 현상이 나타났으며, 이는 기존 실험 연구에서 나온 결과와 유사한 형태였다. 본 연구에서는 개별 세포 수준의 모델링으로 박테리아 집단의 생장을 구현하고, 이를 바탕으로 박테리아의 항생제 내성 현상을 구현하고 예측하였다. 이를 위하여 설계한 박테리아, 항생제, 효소, 환경의 시뮬레이션 모델과 ARSim 프로그램이 실제 임상에서 혹은 기초연구에 종사하는 연구자들에게 항생제 내성을 연구하는데 유용한 도구로 활용되어지기를 바란다.

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주요어 : 항생제 내성, 항생제 내성균, 슈퍼박테리아, 시뮬레이션 바이오인포매틱스, Individual based Modeling

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